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SID 5 Research Project Final Report

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Project identification

1. Defra Project code: CPEA 33
2. Project title: Effects of Ground Level Ozone on Vegetation
3. Contractor organisation(s): Centre for Ecology & Hydrology
4. Total Defra project costs (agreed fixed price): £ 518979
5. Project: start date ..... 01 April 2006
   end date 31/3/2010

6. It is Defra's intention to publish this form.
Please confirm your agreement to do so.....**YES** ☒ **NO** ☐

(a) When preparing SID 5s contractors should bear in mind that Defra intends that they be made public. They should be written in a clear and concise manner and represent a full account of the research project which someone not closely associated with the project can follow.
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(b) If you have answered NO, please explain why the Final report should not be released into public domain

Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

All the experiments conducted in this project use ozone exposures that either are already currently occurring or are expected to occur over UK uplands this century, if predictions of increasing 'background' ozone exposure are correct. Uplands are particularly vulnerable to such increases, because they are windy and wet – conditions which favour ozone uptake by vegetation. Consequently, the observations on ozone effects on upland plant species are likely to occur in the foreseeable future, if they are not already happening.

The novel field ozone exposure system at Keenley Fell (Northumberland) has been operating since spring 2007. Seasonal average increases in ozone exposure at 10 m from the release point were very small in 2007, increasing to around 5 ppb above ambient in 2008 and 2009. The effects of the ozone treatments on this conservation-managed grassland should be seen in the context of this very modest increase in annual mean exposure.

Average deposition velocity for ozone at Keenley Fell was between 1 mm/s (night) and 5 mm/s (mid-day), varying with weather conditions. These flux data will assist with the modelling of ozone deposition to upland grasslands. Limited flux measurements for CO2 showed uptake during the day and release at night, as expected, and will contribute to modelling interactions between ozone and carbon fluxes under these conditions.

The ozone treatments at Keenley Fell had no effect on total above-ground production in any of the three years. However, there was a cumulative decrease in forb biomass, with an associated change in forb species composition, over the three years. This was accompanied by an increase in grass biomass. These effects of ozone are antagonistic to the objectives of the conservation management at this site.

The biomass and flowering of a small number of individual forb species were significantly reduced by ozone exposure at Keenley Fell. Importantly, these species included the hemi-parasite *Rhinanthus minor* which is frequently used to enhance species diversity in this type of conservation management. Hence, the observed effects of ozone on species composition may partly have been caused through its adverse effects on *Rhinanthus* .

Data compiled from several experiments show adverse effects of ozone on semi-natural plant species at concentrations as low as 30 ppb, with 12 of the species studied showing effects at concentrations below 50 ppbv. Effects on roots were greater than on shoots, with potentially significant implications for overwintering, drought tolerance and carbon sequestration.

Native spring bulbs proved sensitive to ozone, with significant carry-over effects from one season to the next in terms of reduced bulb mass. Negative effects on spring bulbs were observed at average ozone concentrations of 30-40 ppb, suggesting that such woodland and hedgerow species may be at significant risk from rising background ozone concentrations experienced across the UK.

In wetland ecosystems, *Sphagnum* (moss) and *Eriophorum* (bog cotton) were relatively insensitive to ozone exposure, but other sedges and rushes showed delayed emergence and enhanced senescence.

In the first year of exposure of simulated BAP Priority Habitats to a range of ozone treatments, enhanced senescence occurred on several forb species plus the ozone-sensitive grasses *Briza media* and *Festuca rubra* in the calcareous grassland and acid heath mesocosms.

After 6 years of experimental treatment, there continued to be negative effects of simulated future ozone climates on the productivity and species diversity of a simulated MG3b grassland community (typical species-rich UK upland meadow community (*Anthoxanthum odoratum-Geranium sylvaticum* [*Briza media* sub-community]; NVC MG3b). Key indicator species continued to decline in abundance, in favour of invasive grasses.

Exploration of below-ground impacts of ozone on the MG3b mesocosms revealed significant impacts of elevated ozone on root biomass in the low fertility treatment, but not at high fertility. There appeared to be no significant effects of ozone on mycorrhizal colonisation, but marked and significant declines in both productivity and diversity of bulk soil fungal and bacterial community communities were observed. These impacts are worthy of further investigation as the effects detected were of a magnitude expected to impact on ecosystem services.

The early stages of a long-term study on a legume-rich fixed sand dune grassland community of high conservation importance (*Ammophila arenaria-Arrhenatherum elatius* [*Geranium sanguineum* sub-community]; NVC SD9b) revealed negative effects of ozone on both productivity and legume content, with *Ononis repens, Lathyrus pratensis* and *Vicia* spp suppressed in favour of several grasses.

Interactions with moderate drought treatments under increasing background ozone implied that ozone interfered with stomatal control mechanisms with thresholds for effects just above the current background; this observation was confirmed by laboratory experiments (below). Effects of ozone were enhanced in water-saturated soils.

Effects on competition under small incremental increases in background ozone showed that ozone exposure favoured the growth of grasses over forbs, a result also observed in the Keenley field experiment and in the MG3b mesocosm studies.

Experiments with peat bog mesocosms confirmed earlier findings that methane emissions were reduced with increasing ozone concentrations, with no evidence of a threshold for effects.

Laboratory studies on mechanisms showed that ozone can affect drought responses of plants by interrupting the within-plant hormonal signalling that closes stomata (and reduces water loss) in response to drought. This leads to effects on growth even in the absence of visible injury. This study has suggested methods for rapid screening of ozone-sensitive plant species, and possible ameliorative action.

Overall policy implications:

There is now evidence that exposure of semi-natural upland grassland communities and species to current UK ozone concentrations has measurable effects, in terms of shifts in species composition, that are likely to increase in future as 'background' ozone concentrations increase further. Many of these effects of ozone are antagonistic to the conservation objectives of management of these communities.

Effects below ground have been shown in many cases to be larger than above ground, so that changes to plant communities may not be immediately obvious. Decreases in root biomass have implications for interactions with drought stress and carbon sequestration. Changes are not restricted to plants - microbial and fungal populations are also affected.

A better understanding of how ozone affects plants, based on the results from field, controlled environment and laboratory studies in this project, has provided information to drive models of future responses, and has also suggested ways in which the adverse effects of increasing ozone might be countered by suppressing the effect of ozone on hormonal signalling in plants. These findings also provide a means of screening plants in the laboratory to determine whether particular species are susceptible to ozone exposure.

Ozone stomatal flux-response functions have been derived from data collected in these experiments and have been used in the development of new critical levels for application in international risk assessments for ozone effects on (semi-)natural vegetation.

Potential effects of ozone on ecosystem services are summarised, with supporting evidence, with effects expected on food, fuel and fibre production, water supply, climate regulation, genetic diversity, pollination and aesthetics.

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:
   - the scientific objectives as set out in the contract;
   - the extent to which the objectives set out in the contract have been met;
   - details of methods used and the results obtained, including statistical analysis (if appropriate);
   - a discussion of the results and their reliability;
   - the main implications of the findings;
   - possible future work; and
   - any action resulting from the research (e.g. IP, Knowledge Transfer).

INTRODUCTION

This project was undertaken by a consortium of researchers at the Centre for Ecology & Hydrology and the Universities of Newcastle, York and Lancaster. It addressed the potential impact of increasing ozone concentrations close to the earth's surface on natural and semi-natural vegetation in the United Kingdom.

BACKGROUND & CONTEXT

European controls on the emissions of pollutants that lead to the formation of ozone (nitrogen oxides and volatile organic compounds, from vehicles and fossil-fuel combustion) have led to a marked reduction in Europe in the worst episodes of ozone pollution. However, there is evidence that industrial emissions worldwide are causing a slow but inexorable rise in 'background' ozone concentrations. Ozone and its precursors can travel around the northern hemisphere before reaching the earth's surface. This increase in 'background' concentrations is of concern because many plant species begin to show adverse effects of ozone exposure at average concentrations not far above those experienced today.

There has been much work done on crop species, in order to estimate yield losses caused by ozone exposure, but there is little knowledge of the potential effects of ozone on natural vegetation. This project was designed to improve our understanding of which native species are most sensitive, and whether the growing conditions have any influence on the results. Recent studies indicated the plant species and natural habitats that might be at greatest risk, leading us to concentrate on grasslands. We have looked at interactions involving how wet the soil is, the availability of nutrients, and whether the plants are growing in a mixture or as a single species. We did this by measuring plant growth, and also some of the fundamental processes involved, such as the rate of exchange of gases through leaf surfaces, as well as effects on plant hormones.

We worked in the laboratory, to test some of the more fundamental processes, and also in open-top chambers and hemispherical greenhouses supplied with different ozone concentrations. We built and operated a novel 'field fumigation' experiment in which natural grassland was exposed to ozone. We measured the rate of uptake of ozone by individual plant species, and in the field. These measurements, and the results of the laboratory and long-term field and chamber experiments, enable us to build computer models that encapsulate the ways in which plants respond to ozone under a range of experimental conditions. These models can then be used to predict, across different areas of the UK, the possible effects of ozone in the future.

Each of the institutions involved brought its own particular expertise to bear on these problems. By working closely together, we have achieved much more than any one research group could do on its own. The project has also served as training for 2 PhD students, who worked between institutions as part of the collaboration.

The project was structured as 5 interlinked Work Packages [WP], each led by a different research group. The work packages were designed to address 8 objectives:

OBJECTIVES

Work packages in parentheses are listed below.

1. To screen a range of semi-natural plant species to assess their sensitivity to ozone, and to identify those most at risk [WP 2.1, 1.2]
2. To develop a field fumigation facility for ozone treatment of semi-natural plant communities in the UK [WP 1.1]
3. To measure changes in species composition of semi-natural plant communities exposed to ozone concentrations typical of those expected in 2050-2080 [WP 1.2, 2.1, 3.1, 4.1, 4.2]
4. To study the growth and physiological responses of a subset of those species to background concentrations of ozone in laboratory experiments [WP 5.1-5.5]
5. To study biological factors and mechanisms which may control the sensitivity and response to ozone concentrations or flux [WP 4.3, 4.4, 5.3, 5.4]
6. To measure ozone fluxes in the field over semi-natural upland vegetation [WP 1.4]
7. To quantify the responses of species and ecosystem processes within semi-natural communities [WP 1.2, 1.3, 2.2, 3.1-3.4, 4.1, 4.2]
8. To bring together the findings under work to meet objective 1 to 7 to produce an assessment of the risk to upland vegetation of the increasing background ozone concentration (final year of contract) [1.5, 2.2, 2.3, 3.2, 4.1, 4.2, 5.6]

WORK PACKAGES

The original 5 work packages were defined as follows, and were modified through the course of the project to include a 4th year of research, in discussion with Defra project officers:

Work Package 1: Response of an ozone-sensitive UK grassland to open-air ozone exposure (CEH Edinburgh)

1.1 To develop a field fumigation facility that provides well-characterised long-term exposure of a semi-natural grassland ecosystem to a spatial gradient of ozone [2]
1.2 To identify the responses of an ozone-sensitive grassland to chronic ozone exposure across a range of concentrations, and any changes in species composition, in conjunction with York University (see 3.1) [3,7]
1.3 To measure physiological and other responses of specific components of the grassland for comparison with responses measured in the solardomes at CEH Bangor, in conjunction with York University (see 3.2) [7]
1.4 To measure the deposition flux of ozone to semi-natural grassland [6]
1.5 To summarise and interpret the overall results of the project in collaboration with other partners [8]

Work Package 2: Predicting the timing of responses of grassland species to the changing ozone profiles of the 21st Century (CEH Bangor)

2.1 To expose over 50 species of grassland, including component species of the Edinburgh and Newcastle communities, to ozone regimes representing current background, 2030, 2060 and 2090 and to quantify sensitivity. [1]
2.2 To determine the factors influencing ozone flux to these species and produce flux-effect models, in conjunction with York University (see 3.2). [6,7,8]
2.3 To develop an ozone-response matrix that indicates the potential timing of shifts in competitive balance within the 21st century. [8]

Work Package 3: Responses of species composition, growth and ecosystem processes to ozone concentration and flux (York University)

3.1 To monitor the impact of ozone on plant growth, productivity and species composition at the field fumigation site (see 1.2) [3,7]
3.2 To make measurements of canopy dynamics, stomatal conductance and ozone concentrations within the canopy at the field fumigation site (see 1.3) and on individual species in solardome experiments (see 2.2), to provide the basis for modelling ozone flux and effects in upland grassland communities [6,7,8]
3.3 To measure the impact of ozone on nutrient resorption, litter production and decomposition in the field fumigation experiment (see 1.1), to assess effects on carbon and nutrient dynamics [7]
3.4 To measure the effects of ozone on species composition, productivity and methane production of peat mesocosms in the Newcastle OTCs (see 4.1) [3,7]

Work Package 4: Assessment and prediction of ozone-induced shifts in species diversity in sensitive UK plant communities (Newcastle University)

4.1 To identify species at greatest risk from exposure to rising background ozone concentrations [3,7,8]
4.2 To quantify the timescales leading to impoverishment of plant communities [3,7,8]
4.3 Examination of the influence of soil nutrient status on ozone impacts [5]
4.4 Assessment of the stomatal conductance and detoxification potential in governing effects [4,5]

Work Package 5: Ozone, water relations and plant productivity (Lancaster University)

5.1 Quantify responses of growth and gas exchange to ozone and/or mild soil drying of species showing extremes of growth sensitivity and insensitivity to ozone exposure [4]
5.2 Quantify the impact of any differences in stomatal response on plant water relations [4]
5.3 Quantify the dose of ozone received by the mesophyll of leaves of different species [4,8]
5.4 Quantify effects of ozone and mild soil drying on hormonal relationships of subject plants (ABA and Ethylene) [4,5]
5.5 Quantify the impact of oxidative stress and mild water deficit on key enzymes in the plant's antioxidant network (e.g. APX2) [4,5]
5.6 Assess the impact of interactions of changing rainfall patterns and increasing tropospheric ozone on productivity and competitive potential of upland plants in the UK [8]

RESEARCH QUESTIONS

The project was designed to answer a series of research questions:

1. What is the minimum concentration/dose of ozone at which significant changes in species composition and adverse physiological and growth effects occur in a range of semi-natural vegetation characteristic of upland Britain? [1.1, 1.2, 1.3, 2.1, 3.1, 3.2, 4.1, 4.2]
2. Which upland species are at greatest risk of effects of increasing background concentration? [1.2, 2.1, 4.1]
3. Are differences in ozone sensitivity between the species studied due mainly to apoplast free radical scavengers? [4.4, 5.5]
4. Are the responses to ozone associated with stomatal uptake? [1.4, 2.2, 3.2, 4.4, 5.3]
5. Are there any effects of non-stomatal ozone deposition? [1.4, 5.3]
6. Where in the UK are the effects of background ozone likely to be greatest? [1.5, 2.2, 2.3, 4.2, 5.6]

RESULTS

In order to achieve Objective 8, we have synthesised the findings from this study in three broad sections, covering in turn:
Development of field fumigation and flux measurements (Objectives 2 and 6)
Effects on individual species, species composition, and ecosystem processes (Objectives 1,3,7)
Physiological and mechanistic studies (Objectives 4 and 5)

A final section briefly considers the policy implications of the work.

Development of field fumigation and flux measurements (Objectives 2 and 6)

Keenley Fell Ozone Field Fumigation Facility (KOFF)

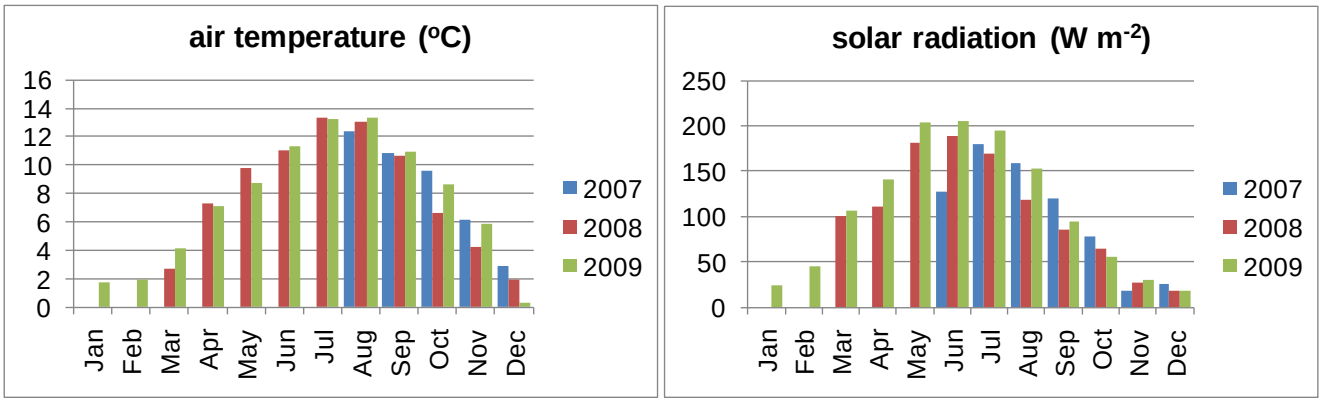
The site is located ~20 km west of Hexham in Northumberland (NY792558) in a field that is managed for its conservation interest. The layout of the site and fumigation system is shown in Figure 1; there are three linear release manifolds that are individually controlled to expose the grassland to ~30 ppb of ozone above the ambient level. In addition to the fumigation control equipment there is also a suite of meteorological and flux measuring instruments. The fumigation system is designed to operate when the wind-direction is from the SW sector (180 to 270 °N), the prevailing wind direction. This occurred for ~63%, 56% and 61% of the time in 2007, 2008 and 2009, respectively. Average monthly weather conditions in each of the 3 years are shown in Figure 2.

Ozone is released independently along each of the 3 replicate transects under real-time computer control, with a 'target' ozone concentration of 20 ppb (30 ppb from 2008) above ambient at a distance of 10 m downwind from the release manifolds. Ozone is generated by electric discharge in oxygen. Control and monitoring of ozone concentrations is achieved by dual-channel UV photometric ozone analysers. The average spatial patterns of ozone exposure along each of the transects are also measured using passive diffusion samplers.

There were several problems in implementing the exposure system in 2007, meaning that the target ozone concentrations were not achieved. Even in 2008 and 2009, the windy nature of the site meant that target concentrations could not be met in the windiest conditions. The average concentrations achieved throughout the period at 10m from the release point (the 'target' distance) and at 5m distance are shown in Figure 3. These distances (10 m and 5 m) are referred to later as "+10 ppb" and "+25 ppb", but it can be seen from Figure 4 that the actual increases in concentrations referred to by the shorthand labels were much lower than suggested by the labels, averaging +1, +5 and +4 ppb, respectively, at 10 m in each of the 3 years.

Measurements of ozone fluxes to the grass field upwind of the treatment area were made using eddy covariance, combining vertical wind speed data measured at 10 Hz with ozone concentrations from a rapid-response ozone analyzer, and an infrared carbon dioxide analyzer. The resultant fluxes are summarised for 2007 and 2008 in Figure 5, and demonstrate the expected increase in deposition velocity during daytime as a result of the stomatal uptake of ozone by the grassland community. The average diurnal pattern of carbon dioxide and latent heat (water vapour) fluxes in 2008 are also shown in Figure 5.

Figure 1: schematic of experimental layout at Keenley Fell field fumigation site.



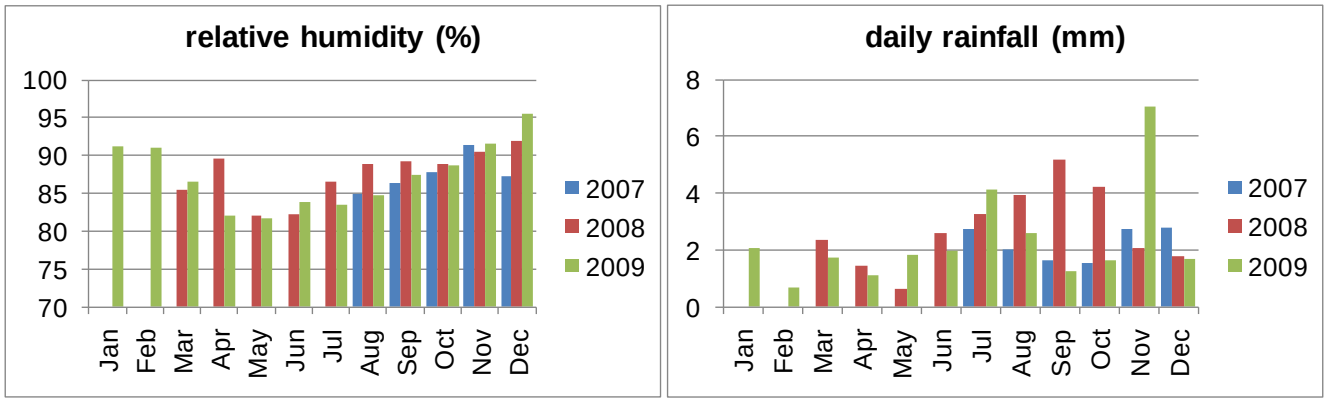


Figure 2: summary of monthly average weather conditions at Keenley Fell, 2007-2009.

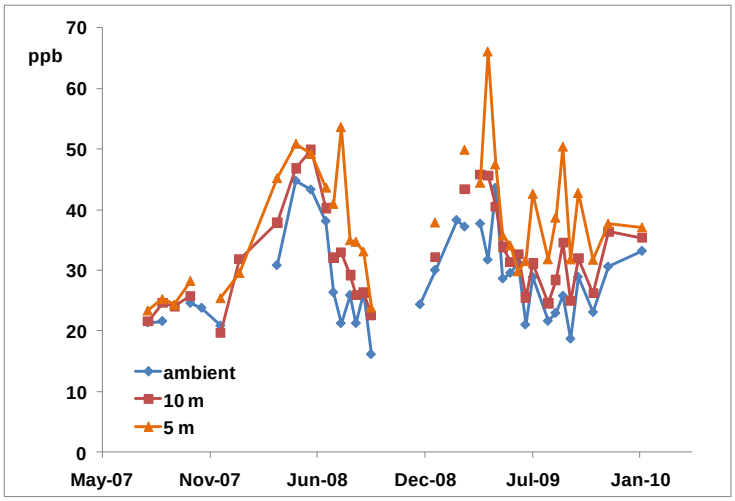


Figure 3. Average ozone concentrations across all transects at distances of 5 m and 10 m from the ozone release points, in comparison with ambient ozone concentrations.

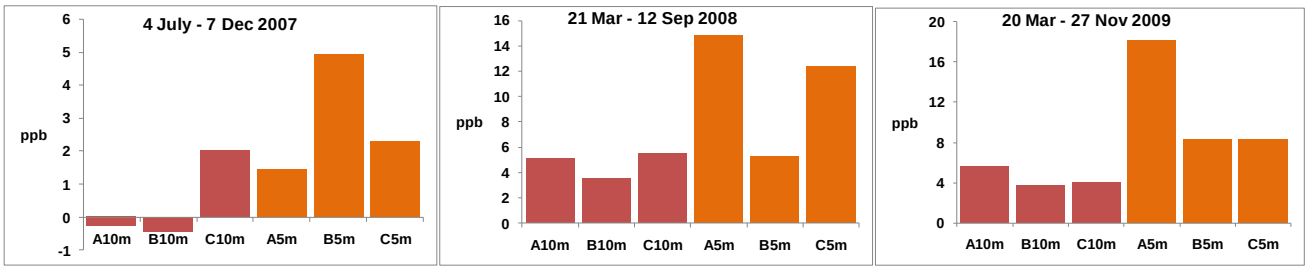


Figure 4. Average ozone enhancement above ambient at 10 m and 5 m from the release point in each growing season, showing variation across the replicate transects A, B and C

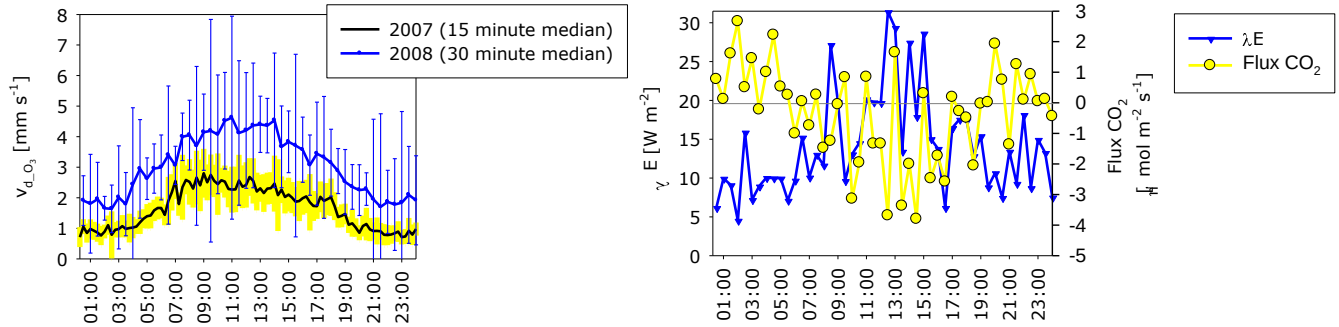


Figure 5. Average diurnal variation in ozone flux to grassland (left), and latent heat (water vapour) and carbon dioxide fluxes in 2008 (right).

Effects on individual species, species composition, and ecosystem processes (Objectives 1,3,7)

Objectives 1, 3 and 7 address effects of ozone pollution on the growth of individual species, on species composition and on ecosystem processes. Three important vegetation types were targeted for investigation: grasslands managed for conservation value, wetlands and woodland-edge spring flora. For each, quantifying the

effects of rising background ozone concentration during this century was the over-riding theme. Experiments were carried out using four different experimental approaches:-

Exposure of individual grassland species to ozone in controlled-environment chambers at Lancaster University, using ambient and 70 ppb as ozone treatments.

Exposure of individual grassland species, mixed species mesocosms, including simulated BAP priority habitats, and cores of wetland vegetation in solardomes at CEH Bangor using treatments simulating increasing background ozone at an upland site (4 treatments in 2006, 8 treatments in 2007 and 2008).

Exposure of individual species (spring flowering bulbs), long-established mixed-species grassland communities (mesocosms) and cores of wetland vegetation utilising 16 open-top chambers at Newcastle University (at 4 ozone levels).

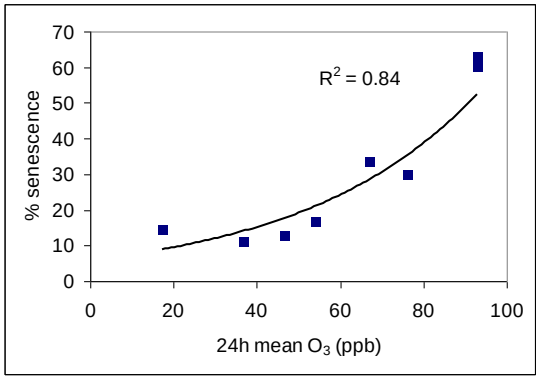
Field fumigation of an intact grassland at Keenley Fell (ambient and ambient plus nominal 5, 10 and 25 ppb) Here we combine the information from these different experimental approaches to assess the evidence that ozone has significant effects at these different levels of ecosystem organisation.

Over the last ten years, international effort has focussed on the establishment of ozone-effect relationships based on parameters that represent either the ozone concentration in air above the canopy (e.g. 24h mean, AOT40) or the stomatal flux of ozone into the leaves. The latter is biologically more relevant since it takes into account the effect of climate, soil and plant parameters on stomatal aperture and gas exchange and has been included in the LRTAP Convention Mapping Manual for crops (wheat and potato) and provisionally for trees (beech and birch). Derivation of flux-effect relationships for mixed-species natural or (semi-)natural vegetation communities is more complex and is included in two other DEFRA contracts (AQ 0810, AW0601PP). Herein, we present flux-effect relationships for single species and plant communities, with a key objective of the study to identify thresholds for effects of increasing background ozone on key species. For comparison across the different experiments, effects are mainly analysed in relation to the 24h or exposure period mean ozone concentration. This ozone parameter was selected because AOT40 is not applicable to studies probing the effects of increasing background levels and/or upland environments where ozone concentrations exceeded 40 ppb almost all of the time - even in some of the lowest treatments - and where effects have been detected in some species at concentrations below 40 ppb.

Effects on individual species - responses detected within one season in a competitive environment

In all of the following experiments, several plants of each species were grown in large containers in competition with plants of a species with which it normally co-exists in the field, to provide a more realistic growing environment than using a single species alone. The solardome facility at CEH Bangor facilitated the study of effects of small increments in ozone above and below the current ambient, and by careful hand watering enabled responses to water regimes predicted to be more frequent in the future (e.g. reduced watering, occasional waterlogging) to be studied.

(a) Responses to ozone with adequate water supply.
The cost of maintaining shoot growth in a senescing canopy is illustrated in Figure 6 for *Dactylis glomerata* (growing in competition with *Ranunculus acris*) exposed to eight ozone treatments in the range 17 to 90 ppb ozone for 20 weeks in the solardomes at CEH Bangor in 2008. Eight weeks after the start of the ozone exposure, there was an exponential increase in senescence with increasing ozone concentration, reaching 60% of leaves for the two highest treatments (Figure 6a). The foliage was cut-back to 7 cm after 12 weeks to simulate a hay-meadow cut; the biomass of the cut foliage showed a small but significant increase with increasing ozone exposure. By the end of the 20-week experiment, ozone exposure resulted in no significant change in above ground biomass whilst the roots were substantially smaller in the highest ozone treatments (almost 50% smaller for the 89 ppb treatment than those of the 34 ppb, simulated ambient, treatment). Such a marked effect on root growth has major implications for overwintering, drought tolerance and carbon sequestration.



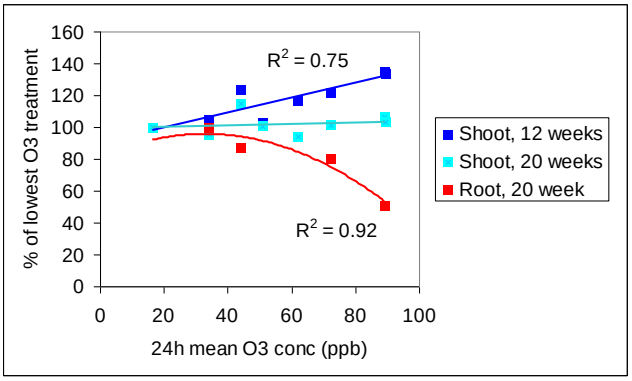


Figure 6. The effect of increasing background ozone on (a) senescence after 8 weeks exposure and (b) above ground biomass of *Dactylis glomerata* (when grown in competition with *Ranuculus acris*) after 12 weeks, and above and below-ground biomass at the final harvest (after 20 weeks).

(b) Responses with modified water supply to simulate either a mild drought or waterlogging.
In addition to the adequate watering treatment described above, ozone effects on the *Dactylis glomerata: Ranunculus acris* mesocosms were also assessed in 2008 for an additional moderately reduced watering treatment. Although the plants from the reduced-watering treatment grew larger roots than those from the field capacity treatment (presumably in order to maximise water resource utilisation), there was still a reduction in root growth in response to ozone (Figure 7b). Over 800 stomatal conductance measurements were made during the season, and for both species soil water content was an important factor determining conductance and hence ozone flux during daylight hours (Figure 7a). After 20 weeks of ozone exposure, root biomass was strongly correlated with ozone flux (r2 = 0.88, Figure 7b). For each of the four ozone treatments studied, the modelled ozone flux was lower and root biomass was higher for the reduced water compared to the field capacity treatment. However, there was a marked change in the slope of the response function as ozone fluxes/ozone exposure increased. Thus at moderate reductions in water availability and ozone exposure, reduced stomatal conductance of ozone (and hence flux) reduced the magnitude of ozone damage whilst in the higher ozone treatments, this effect was less pronounced. The latter was most probably due to ozone-induced loss of stomatal control as discussed later (objectives 4 & 5) and published in Mills et al. (2009) and Wilkinson and Davies (2009).

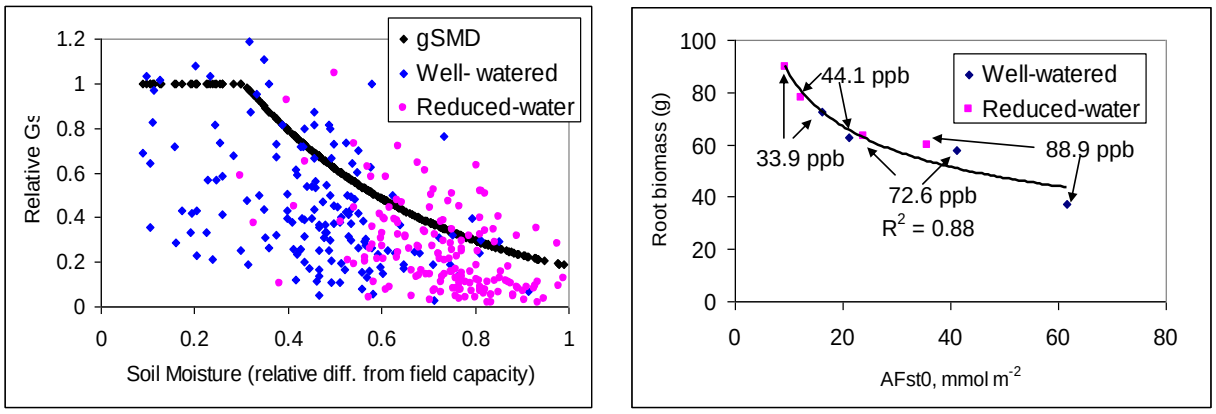


Figure 7. (a) Relationship between stomatal conductance and soil water content for *Dactylis glomerata* and (b) ozone flux-effect relationship for effects on the roots of *Dactylis glomerata*. Note: the concentrations marked on (b) represent the seasonal 24h mean values for the indicated points.

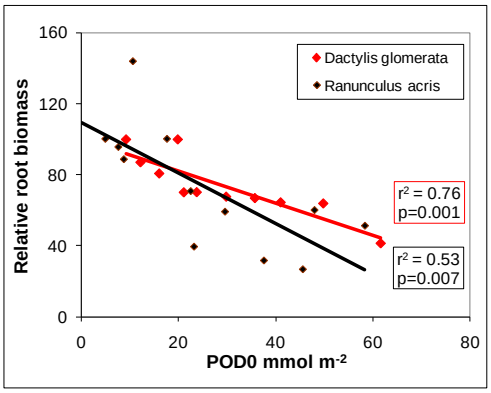
Further two-species competition experiments were conducted in 2009 at CEH Bangor. These experiments were primarily conducted to assist in the interpretation of results from the field-release site at Keenley (see above) where a soil moisture gradient coincides with the ozone gradient on site. Two-species mesocosms were established in April and exposed to ozone for 8 weeks from 20th May to 15th July. *Ranunculus acris* (Meadow Buttercup, forb) was grown in competition with *Dactylis glomerata* (Cocksfoot, grass) in one set of mesocosms whilst *Carex ovalis* (oval sedge) was grown in competition with *Potentilla erecta* in the other set of mesocosms. Water was applied by hand to achieve 30% soil moisture (field capacity), 20% soil moisture (reduced watering) and 50% soil moisture (waterlogged). These were exposed in the CEH Bangor solardomes to eight rural ozone profiles based on an episode at Keenley in May, 2008 in which there were peaks of +10 to + 25 ppb on four days followed by three days of low peaks (ca 5 ppb) superimposed on a background of ca 45 ppb. One treatment simulated this episode, and the other seven increased or decreased the episode ozone concentrations by -30, -20, -10, +10, +20, +30 and +40 ppb.
Throughout this experiment, over 750 stomatal conductance measurements were made for use in the development of flux models. It has been found that in these and other species tested, there is considerable variability in the response of stomatal conductance to soil water content (Table 1), with some having a low minimum and thus having conductance in very dry conditions (e.g. *D. glomerata, C. ovalis*) whilst others had a minimum soil moisture content for conductance of over 10% (e.g. *P. erecta*). Similarly, the soil moisture content resulting in maximum stomatal conductance varied between species from 21%for *Briza media* to 39% for *Carex ovalis*. These differences in sensitivity to soil water content have implications for the interpretation of results from Keenley where a soil moisture gradient co-exists with the ozone gradient.

Table 1: Minimum and maximum soil moisture content (% V/V) required for stomatal conductance for a range of grassland species.

Figure 8. Root biomass (as a percentage of the control biomass for that species in that year) in relation to Phytotoxic Ozone Dose (POD0, mmol m-2) for *D. glomerata* and *R. acris* in both 2008 and 2009 experiments.

Species	Min*	Max**
Briza media	4.2	20.6
Campanula rotundifolia	2.4	20.4
Carex ovalis	0	39.1
Dactylis glomerata	0	37.7
Helianthemum nummularium	15.7	37.0
Lotus corniculatus	9.9	33.6
Potentilla erecta	11.6	25.5
Ranunculus acris	0	36.6
Sanguisorba minor	6.7	22.5
Scabiosa columbaria	5.7	21.1

*Soil moisture where minimum stomatal conductance is reached
**Soil moisture where maximum stomatal conductance is reached



Although not yet tested statistically, there appeared to be a more than additive effect of ozone and waterlogging with a big increase in senescence corresponding to very little increase in flux. Compared to the field capacity treatment, decreased watering reduced the ozone flux and reduced the consequent effect of ozone. When data from 2008 and 2009 were combined for *D. glomerata* and *R. acris* under all watering conditions, there was a linear relationship between ozone flux and root biomass (relative to the lowest treatment) for both species (Figure 8), with *R. acris* being the more sensitive. These results again indicate that the roots of common grassland species, including those present at Keenley, are very sensitive to ozone exposure with implications for carbon sequestration and drought tolerance. Another effect detected that is of potential ecological significance was an ozone-enhanced flowering in *P.erecta* (from 50 per pot in the lowest ozone treatment to 80 per pot in the highest ozone treatment) with this increase present in all three watering regimes. This is a common stress response in flowering plants and indicated that the plants were responding to enhanced ozone stress by increasing their potential to reproduce.

Studies conducted on wetland mesocosms suggested that the species present in these communities are generally rather resistant to ozone. In studies conducted by both York University and CEH Bangor no significant effects were found on the above-ground growth of the two dominant species (*Eriophorum vaginatum* and *Sphagnum papillosum*). In the case of the Newcastle-based studies these effects remained after 3 years' exposure to high ozone exposures (38-60 ppb above ambient during summer months). The lack of response of *Eriophorum* is consistent with the work of Morsky et al. (2008) in Finland. Some previous studies have reported negative effects of high level ozone exposure on *Sphagnum* mosses, and it could be noteworthy that Potter et al. (1996) classified *Sphagnum papillosum* as being relatively resistant to ozone compared to other *Sphagnum* species. Interestingly, however, methane emissions were significantly and consistently reduced by ozone over the course of several seasons, and this could have major implications for climate change modelling scenarios (see below). In contrast, wetland mesocosms dominated by sedges and rushes were exposed to ozone at CEH Bangor by a NERC-funded PhD student in 2007 and 2008. The higher plants of these communities exhibited reduced emergence and growth as well as ozone-induced senescence.

Given the likelihood of future increases in springtime ozone exposure across the UK and the paucity of relevant data on species growing in springtime, an experiment was undertaken to explore the impacts of ozone on several UK woodland/hedgerow spring bulbs of high conservation importance and aesthetic value. Studies conducted in the Newcastle-based OTCs revealed linear exposure-response relationships on bulb weight for *Allium ursinum* (Wild Garlic), *Frittilaria meleagris* (Frittilary), *Hyacinthoides non-scripta* (Wild Bluebell), *Narcissus pseudonarcissus* (Wild Daffodil), *Ornithogalum umbellatum* (Star-of-Bethlehem) and *Tulipa sylvestris* (Wild Tulip). Moreover, when pots were removed (in late June 2007) from the OTCs and placed in cold frames to die back, overwinter, reflower and die-back before excavating the bulbs there was clear evidence of negative carry-over effects of elevated ozone from one year to the next (see below). Exposure-response relationships for bulb biomass are shown in Figure 9; average 24h mean concentrations resulting in significant reductions in bulb biomass were calculated to be 30-40ppb indicating this vegetation category to be particularly sensitive to rising ozone concentrations with *Frittilaria meleagris* (Frittilary), *Ornithogalum umbellatum* (Star-of-Bethlehem) and *Tulipa sylvestris* (Wild Tulip) especially sensitive.

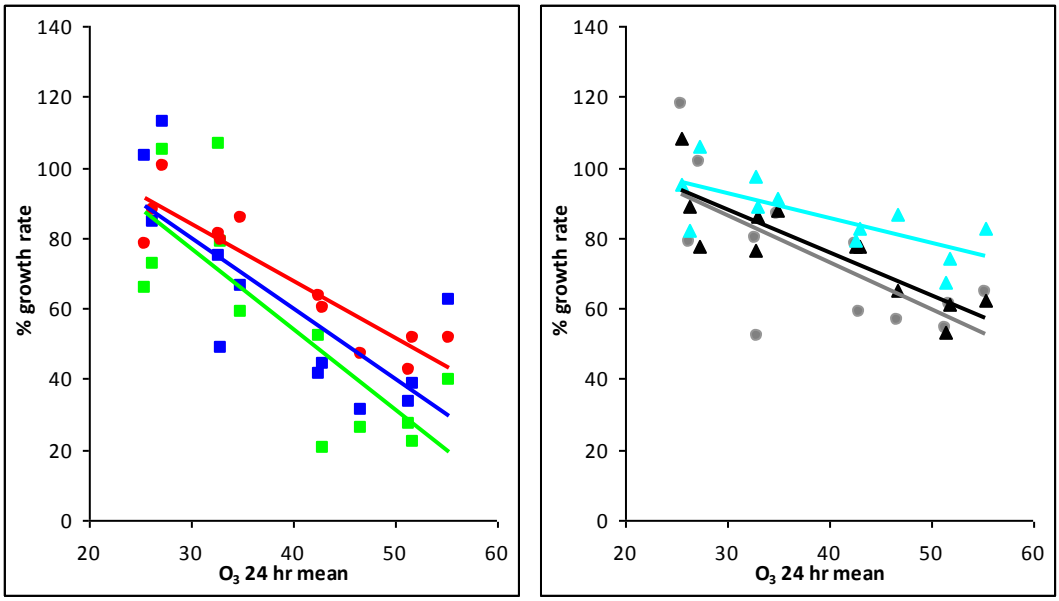


Figure 9: Impacts of increasing background ozone on the growth rate relative to a 20 ppb threshold of the bulb component of six native semi-natural spring bulb species after a 120 day exposure period. a) *Ornithogalum umbellatum* (red line) r2 = 0.83, P<0.001; *Tulipa sylvestris* (dark blue line) r2 = 0.60, P<0.01; *Frittilaria meleagris* (green line) r2 = 0.63, P<0.01. b) *Narcissus pseudonarcissus* (light blue line) r2 = 0.53, P<0.01; *Hyacinthoides non-scripta* (black line) r2 = 0.60, P<0.001; *Allium ursinum* (grey line) r2 = 0.49, P<0.05.

Summary of effects on individual species

A summary table of thresholds for effects of ozone on individual species is shown in Table 2.

Table 2: Threshold concentrations for effects on vegetation

Concentration range where threshold for significant effect detected (relative to <20 ppb O3)	Enhanced senescence After 7-8 weeks of exposure (24h mean)	Negative effects on roots (R) and/or root:shoot ratio (R:S) (24h mean)	Threshold for effects on stomatal control (see footnote)	Threshold for effects on spring bulb biomass (24h mean conc.)
Source of data	CEH Bangor	CEH Bangor	Lancaster & CEH Bangor	Newcastle University
20 – 30 ppb				Ornithogalum umbellatum (29 ppb)
30 – 40 ppb	Luzula campestris (30 ppb)			Allium ursinum (36 ppb) Frittilaria meleagris (33 ppb) Hyacinthoides non-scripta (31 ppb) Narcissus pseudonarcissus (37 ppb) Tulipa sylvestris (34 ppb)
40 – 50 ppb	Viola riviniana (44 ppb) Ranunculus acris (43 ppb) Lathyrus pratensis (46 ppb) Potentilla erecta (47 ppb) Anthox. odoratum (48 ppb)			
50 – 60 ppb	Succisa pratensis (50 ppb) Molinia caerulea	Ranunculus acris (R:S 55 ppb, R, 57 ppb)	Dactylis glomerata1 (57 ppb)	

Concentration range where threshold for significant effect detected (relative to <20 ppb O3)	Enhanced senescence After 7-8 weeks of exposure (24h mean)	Negative effects on roots (R) and/or root:shoot ratio (R:S) (24h mean)	Threshold for effects on stomatal control (see footnote)	Threshold for effects on spring bulb biomass (24h mean conc.)
	(50 ppb) Juncus nodulosus (54 ppb) Leontodon hispidus (59 ppb)			
60 – 70 ppb	Carex flacca (67 ppb)	Dactylis glom. (R : S, R, 66 ppb)		
70 – 80 ppb		Carex flacca (R:S, 71 ppb)	Responses at 70 ppb: Leontodon hispidus (1,2,3,4) Rumex acetosa (2) Ranunculus acris (2) Anthox.odoratum (1) Lolium perenne (1) Trifolium repens (1) D. glomerata ((2), 76 ppb)	
80 – 90 ppb		Leontodon hispidus (Rt, 86 ppb)		
90 – 100 ppb		Molinea caerulea (R:S, 91 ppb) Anthox. odoratum (R:S, 92 ppb)		

Effects on stomatal control were: (1) reduced ability to withstand water stress, (2) increased stomatal conductance (3) reduced sensitivity to ABA and (3) increased ethylene emission

Responses detected over multiple seasons

A long-term study (now in its sixth consecutive year) on the impacts of ozone on MG3b mesocosms (24 mesocosms per treatment in 40 dm3 containers exposed in duplicate OTCs) containing a typical species-rich UK upland meadow community (*Anthoxanthum odoratum-Geranium sylvaticum* [*Briza media* sub-community]; NVC MG3b) reveals a steady and cumulative effect of ozone exposure on above-ground productivity (Figure 10).

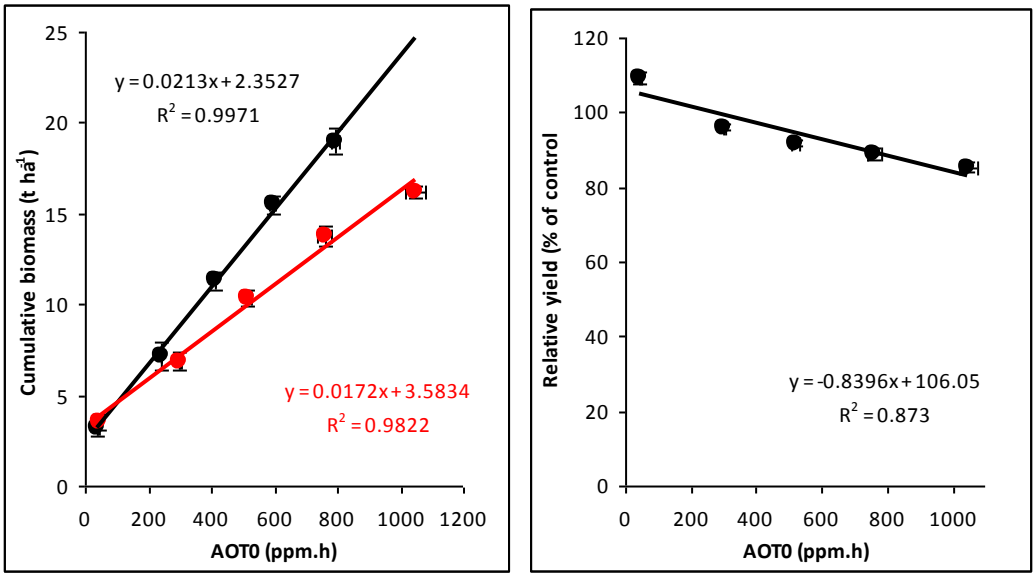


Figure 10. Impacts of simulated present-day (black line) versus 2050 UK-upland ozone climate (red line) on a mesotrophic grassland community. a) total cumulative above-ground biomass (P <0.01) and b) impacts relative to yield of control (P <0.05).

Carry-over effects from one year to the next

The spring bulb experiment at Newcastle was designed to explore carry-over effects of ozone treatment on spring bulbs, from one season to the next. Two of the species tested (*Tulipa sylvestris* and *Frittilaria meleagris*) showed

clear exposure-response carry-over effects from one season to the next, with reduced growth rates in the subsequent season even without further exposure to ozone. Carry-over effects were also detected during studies on mesocosms populated with two target species during ozone exposures in the solardomes at CEH Bangor. Pots containing *Leontodon hispidus* in competition with either *Anthoxanthum odoratum* or *Dactylis glomerata* were exposed to 8 ozone treatments for 20 weeks (in 2007) and then left to overwinter outdoors; the *L. hispidus* : *A. odoratum* mesocosms were subsequently exposed to a second season of ozone exposure (see below). In the autumn, *L. hispidus* exhibited reduced die back of green leaves, with the effect linearly related to the previous spring/summer ozone exposure. Reduced root growth was still present in the highest ozone treatments the following spring, and in July mesocosms from the higher ozone treatments had a lower flower: seed head ratio suggesting earlier flowering in the higher ozone treatments. For the *A. odoratum : L. hispidus* communities exposed over two seasons, negative effects of ozone on above-ground biomass for *L. hispidus* were only apparent in year 2.

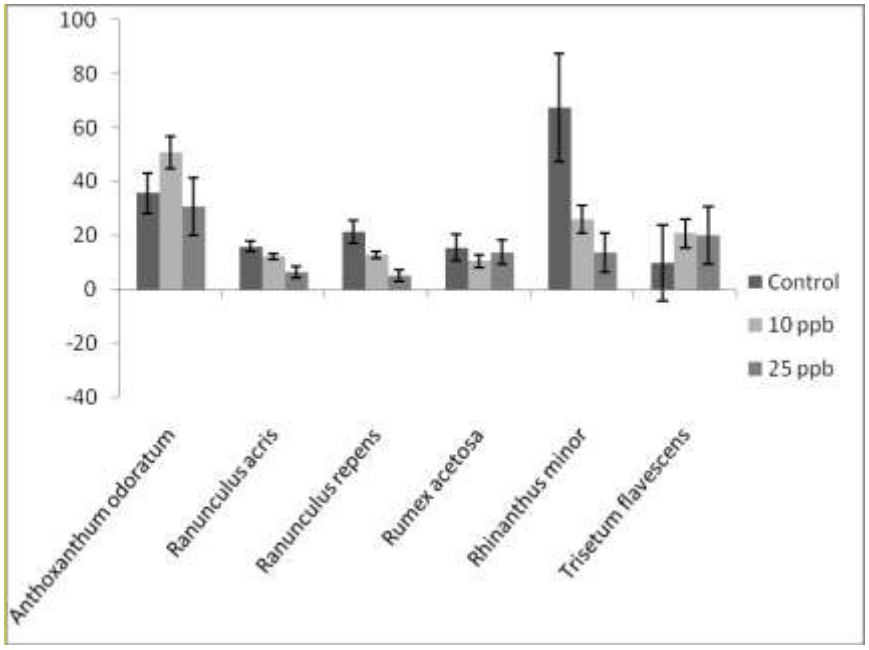
Effects on species composition/diversity

Ozone effects on species composition have been examined at different scales under this contract. At Keenley Fell ozone effects on upland grassland community dynamics have been explored by York University. At Newcastle University, long-term ozone effects on competition have been investigated using mesocosms representing MG3b and SD9b grassland communities of high conservation value, and at CEH Bangor, studies have focused on factors affecting above and below ground competition (strength of competition, water availability, changes with time).

Effects on biodiversity measured at Keenley Fell

Assessment of the cover and flowering of individual species
In 2007 and 2008, assessment of the effects of ozone focussed on a destructive harvest in August. The objective of the 2009 study was to assess impacts of ozone on the grassland community earlier in the growing season. Because of the importance of flowering to the improvement in biodiversity in this upland ecosystem, and the ecosystem services that are provided by flowering, we focussed on this response. We carried out detailed assessments of flowering over the spring (13th-20th June 2009) and early summer (21st to 24th July 2009), in order to capture any ozone effects on both early- and late flowering species. Rather than following a small number of species in detail, we conducted broader surveys of flowering of all species, in order to assess if ozone effects on flowering were consistent with those observed on biomass production.

The results are summarised in Figure 11 for the species that dominated flowering in the two assessment periods; for many species, only a small number of flowers were observed, making any evaluation of treatment effects impossible. In June, there was a clear trend of decreasing flower numbers for *Rhinanthus minor* and for the two *Ranunculus* species. There was no clear effect of treatment on the two grass species or on *Rumex acetosa*. In July, there was evidence of increased flower numbers for *Poa trivialis* in the elevated ozone treatment, but no effect on *Holcus lanatus.*



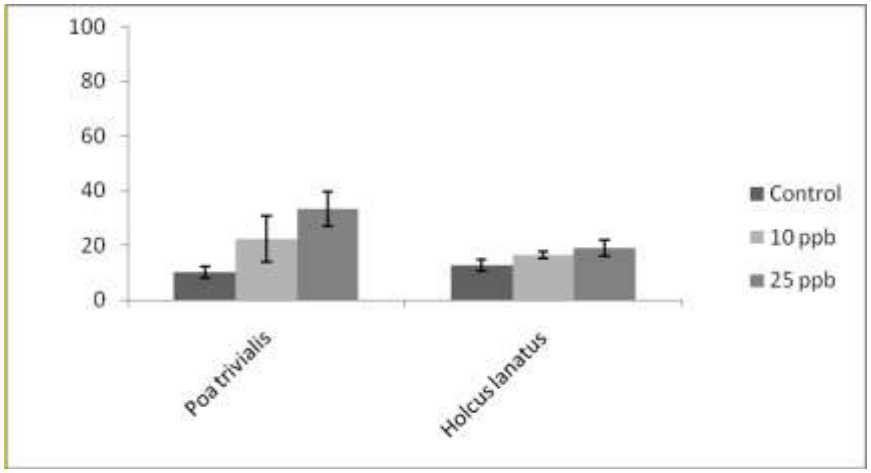


Figure 11.. Summary of effects of ozone on flowering in June and July 2009. Only data for species with more than an average flower count of 10 m-2 are shown. The bars represent standard errors of the mean flower counts. The different treatments refer to nominal rather than actual concentration enhancements (see Figure 4).

Effects of ozone exposure on biomass

A harvest of above-ground biomass was conducted in early August 2009, quantifying total forb, legume and grass biomass, and that of individual species. This followed the same protocol as in the previous harvests, in August 2007 and 2008. In the previous report, after two years of the project, we commented that it was uncertain whether the apparent treatment effects on the forb and grass components were really due to the ozone treatment. This was because of the spatial variation in initial species composition and in soil characteristics. Putting the results for 2009 in the context of the data for 2007 and 2008 provides much greater confidence that this is the case, for reasons explained below. Therefore, in order to understand the cumulative effects of ozone over the period of this project, and the period of operation of the Keenley Fell site, data for all three years have been combined in this report.

The mean total above-ground biomass showed no significant effect of ozone treatment in any of the three years. However, it did show a significant gradient across the site in 2007 and 2009, with biomass in each transect being different from the other two. In all three years, there was a clear trend for the highest total biomass to be found in Transect 3, with the lowest biomass in Transect 1. The biomass of legumes and grasses also tended to show greater values in Transects 3 and 2 than in Transect 1, which was consistent with the gradient of soil fertility overall across the site. However, no such effect was found for forb biomass. Grass biomass was not significantly affected by ozone in 2007 or 2008 but a significantly greater biomass was found in ozone-treated plots than in the control plots in 2009.

In 2007, when very little ozone exposure had occurred, there was no significant effect of ozone on forb biomass. However, in both 2008 and 2009, both elevated ozone treatments significantly reduced the forb biomass. There is evidence that the size of this effect of ozone increased over the three years of the experiment (Figure 12). It is also important to note the trend in forb biomass over time. In the control treatment, the forb biomass has increased over the course of the experiment. Since this would be one objective of the environmental stewardship management that is prescribed at the site, this could represent part of a longer-term trend due to the change in management. However, there is no such trend apparent in the ozone treated plots, suggesting that ozone is preventing the management prescription having the planned beneficial effect.

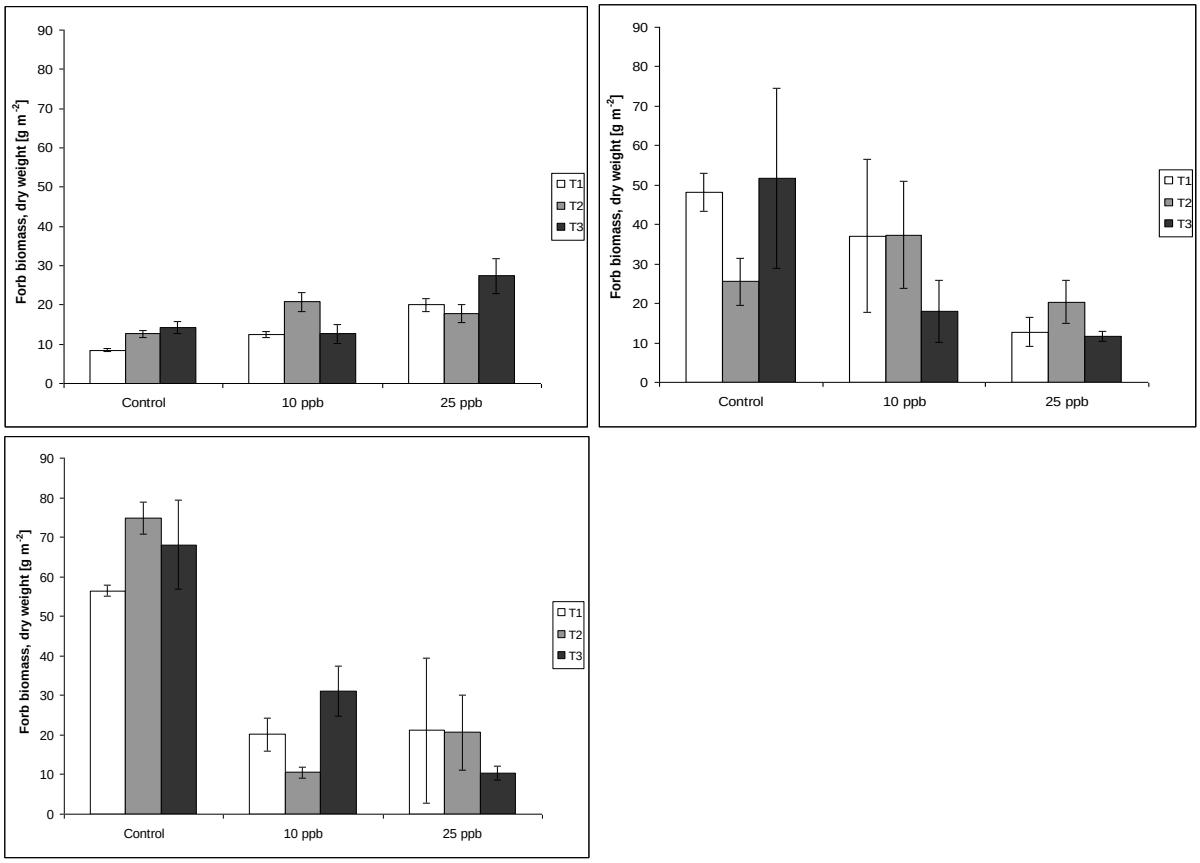


Figure 12. Results of the forb biomass in 2007 (upper left), 2008 (upper right), and 2009 (lower left). Error bars represent the standard error between replicate sub-plots.

For the legumes, on the other hand, biomass was significantly lower in the control plots than in the 10 ppb plots in 2008, and significantly lower in the 10 ppb and 25 ppb plots than in the control plots in 2009. However, in 2009, there was a significant ozone/transect interaction term, showing that the effects of ozone varied between the three transects. Hence, it is not possible to make any firm conclusions about the effects of ozone on legumes, which only represent a relatively minor component of the canopy at the site.

The effect of ozone and transect on the biomass of individual species was also investigated. However, in both 2008 and 2009, the very high variability between plots meant that few significant effects were found. In 2009, at P=0.05, there was a significant negative effect of ozone only on the biomass of *Rhinanthus minor* and *Ranunculus acris*, while a positive effect of ozone was found on *Vicia cracca*. The negative effects on *Rhinanthus minor* are particularly important because of the role of this species in enhancing species diversity in managed grasslands.

Detailed statistical analysis

The variation in soil conditions across the site at Keenley Fell before the start of ozone treatment makes it difficult to separate the effects of ozone and soil conditions. Furthermore, analysis of individual species data in isolation did not provide a very clear analysis of how species composition was responding to ozone. In order to assess the extent of change in species composition within the major functional groups, two multivariate (RDA) analyses were conducted for each year, one with only O3 concentration as an environmental variable, the second with all environmental variables. Soil data measured at 0-10 cm was used in this analysis, alongside ozone treatment and transect.

The analysis was conducted separately for grasses, and for forbs and legumes combined. With O3 as the dominant variable, O3 in all three years only accounted only for a small and non-significant proportion (4-6%) of the variation in grass species composition, which was not significant. In contrast, in the case of forbs, the percentage of variation explained by O3 increased over time, from only 4.0% (P<0.2) in 2007, to 9.5% (P<0.01) in 2008, and 40.3% (P<0.001) in 2009. Hence, much more of the variation in species composition within the forb and legume group can be attributed to O3 in 2009 than in 2008 and in 2007.

Effects measured during one or two seasons of ozone exposure

A recent study (Morrissey et al., 2007) indicated that each of the BAP priority habitats occurring in England is in areas where the April – September AOT40 is relatively high, with 72% of lowland calcareous grassland, 64% of lowland meadows and 60% of fens occurring in regions where the AOT40 was greater than 6.5 ppm h (averaged over 1999 – 2003) and well above the critical level of 5 ppm h. However, studies included here together with

those also included in RoTAP, have shown that AOT40 is no longer fit for purpose. As no flux-based assessment is yet possible for BAP priority habitats, experiments initiated in 2009 at CEH Bangor were designed to begin to fill this gap in knowledge. In the first season, mesocosms representing two BAP Priority Habitats were established using plants from specialist wild-flower nurseries: Lowland calcareous grassland (2 grass and 5 forb species) and Upland heath (1 grass, 1 forb and *Calluna vulgaris*). These were exposed to eight rural ozone profiles based on an episode at Keenley in May, 2008 (see above), providing 24h mean ozone concentrations of 16 ppb, 23 ppb, 36 ppb, 45 ppb, 51 ppb, 61 ppb, 73 ppb and 80 ppb. The mesocosms were established in May and exposed to ozone for 12 weeks from 22nd July to 14th October. Watering was applied by hand to achieve field capacity and ca. one third less water availability (32% and 21% soil moisture content for the well-watered and reduced-watered calcareous grassland respectively, and 40% and 30% soil moisture content for the well-watered and reduced-watered upland heath respectively). Stomatal conductance measurements were made on component species to facilitate the development of flux-effect relationships, together with non-destructive measurements of leaf area index, senescence, green leaf area (using a spectroradiometer, PP Systems) and ground cover. Continuous ozone measurements were made above and within the canopy for representative calcareous mesocosms in the simulated Keenley and Keenley+30 ppb treatments as a way of validating community-scale flux–effect relationships. All of the mesocosms are currently being over-wintered outdoors and will be exposed to a second season of ozone in 2010, starting in late April.

a) Calcareous grasslands

Ozone damage (symptoms and/or enhanced senescence) was detected on *Festuca rubra, Briza media, Sanguisorba minor, Scabiosa columbaria* and *Lotus corniculatus*, with severity of symptoms increasing with increasing O3 concentration. *F. rubra* (red fescue, grass), *S. minor* (salad burnet, forb) and *S. columbaria* (small scabious, forb) were the most sensitive, with a response threshold of 30-40 ppb, which is within the UK ambient O3 range. Leaf area index increased with increasing ozone concentration, indicating an initial burst in above-ground growth in response to ozone, as predicted by Davies and Wilkinson at Lancaster (see below). Reducing the mean soil moisture content from 32% to 21% offered some protection against ozone damage by reducing stomatal conductance, with the extent of protection varying between species and corresponding to a 70, 21 and 36% reduction in senescence in *F. rubra, S.minor* and *S.columbaria* respectively. When the data were combined for the two watering regimes, there was a decrease in the ground cover of the grass species, *Briza media*, a species also found to be declining in the mesocosms at Newcastle. This was associated with an increase in the *F.rubra: B.media* ratio. A more representative indication of the effect of small increases in background ozone concentration on the balance of different species will be found at the end of the second period of ozone exposure, to be conducted in spring/summer 2010. A preliminary conclusion from the first season of ozone exposure is that lowland calcareous grassland is sensitive to ozone and may be at risk of damage in current and near-future profiles.

b) Upland heath

By the end of the first period of ozone exposure, *Calluna vulgaris* (common heather) showed no visible effects of the pollutant. The two other species present with *C. vulgaris* showed increasing senescence with increasing ozone, with *F.rubra* being more sensitive than *Potentilla erecta* (Tormentil).

c) Two species competition experiments

In all the two-species communities exposed to ozone at CEH Bangor in 2007, the percentage of senescent leaves on low-growing *Leontodon hispidus* increased with ozone exposure/concentration. The plants most severely affected were those grown in competition with the shorter-growing *Poa trivialis*, which had the most open canopy (Figure 13a). Effects on *L. hispidus* in competition with the more vigorous *Anthoxanthum odoratum* were less pronounced and the least effects on *L. hispidus* were found when it was growing in the largest and most dense canopy i.e. with *Dactylis glomerata*. These results suggest that ozone flux to low-growing species is influenced by the density of the surrounding canopy, a factor that needs to be taken into account when considering the development of canopy flux-effect relationships. Ozone exposure also modified the competitive balance between species over a two year period. By the end of the second year of exposure, the ratio of above-ground biomass of *L. hispidus* to *A. odoratum* decreased from 4.0 to 0.9 dependent upon O3 exposure concentration between an average 24h exposure of 44 and 73 ppb (Figure 13b). In addition, the root:shoot ratio for *A. odoratum* decreased from 2.5 at 20 ppb ozone to 1.0 at 90 ppb O3 whilst that for *L. hispidus* showed only a slight and non-significant increase with increasing ozone treatment.

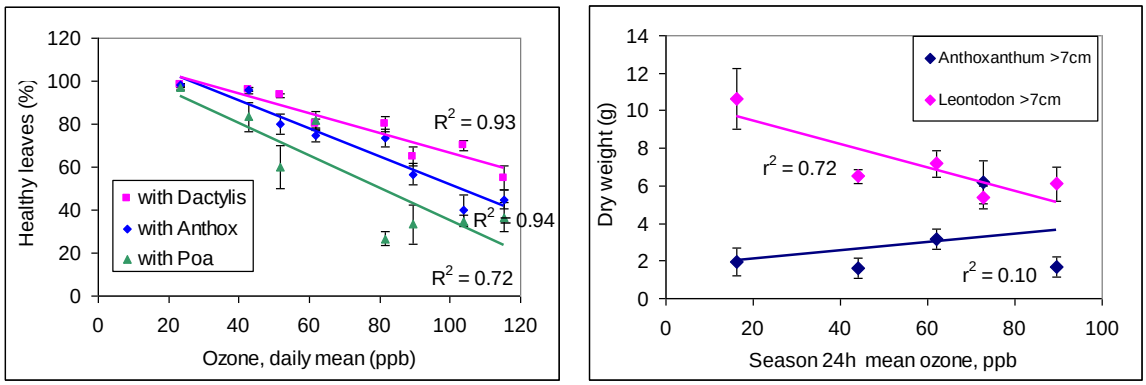


Figure 13. (a) Proportion of healthy leaves on *L. hispidus* after 7 weeks exposure to increasing background ozone, in competition with *D. glomerata, A. oderatum* and *P. trivialis* (b) Effect of increasing background ozone on the competitive balance of *A. odoratum* and *L. hispidus*, expressed as the dry weight of the foliage above 7cm at the end of the second 20 week exposure period.

d) Legume-rich sand dunes

The second year of a study exploring the effects of elevated ozone on a legume-rich SD9b sand dune community at Newcastle revealed dramatic changes in floristic composition with productivity of several key legume species (especially *Ononis repens, Lathyrus pratensis* and *Vicia* spp) suppressed in favour of several grasses (Figures 14 and 15).

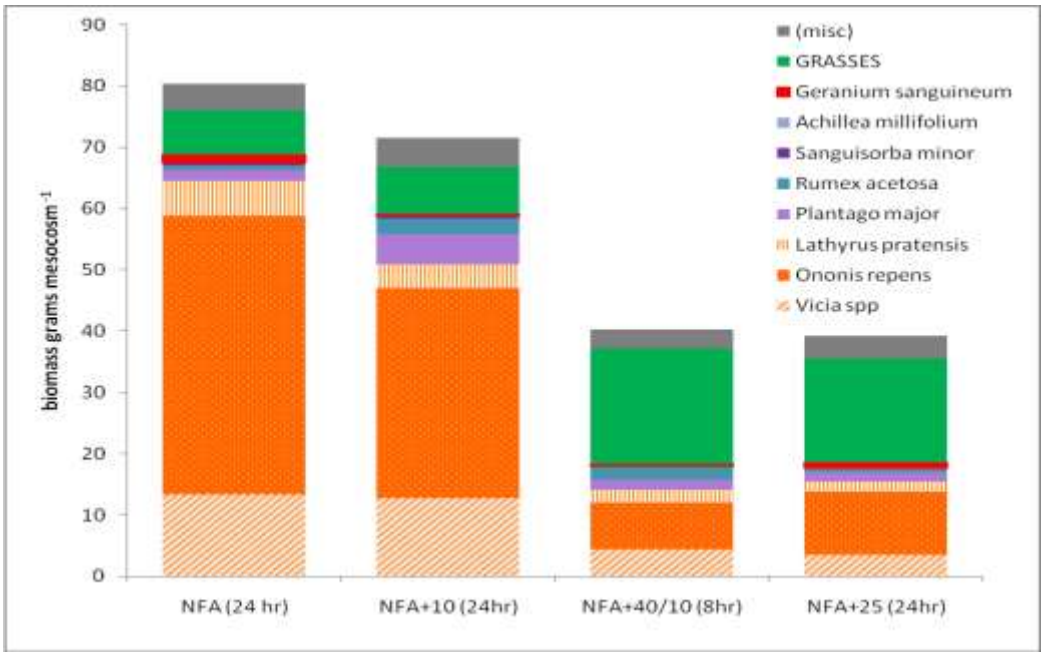


Figure 14. Impacts of elevated ozone on the productivity of a simulated SD9b sand dune community following 900 days' exposure to a) NFA 24 h/day b) NFA+10 ppb ozone 24 h/day c) NFA + 40 ppb ozone (spring-summer)/+10 ppb ozone (autumn-winter) 8 h/day d) NFA + 25 ppb 24 h/day.

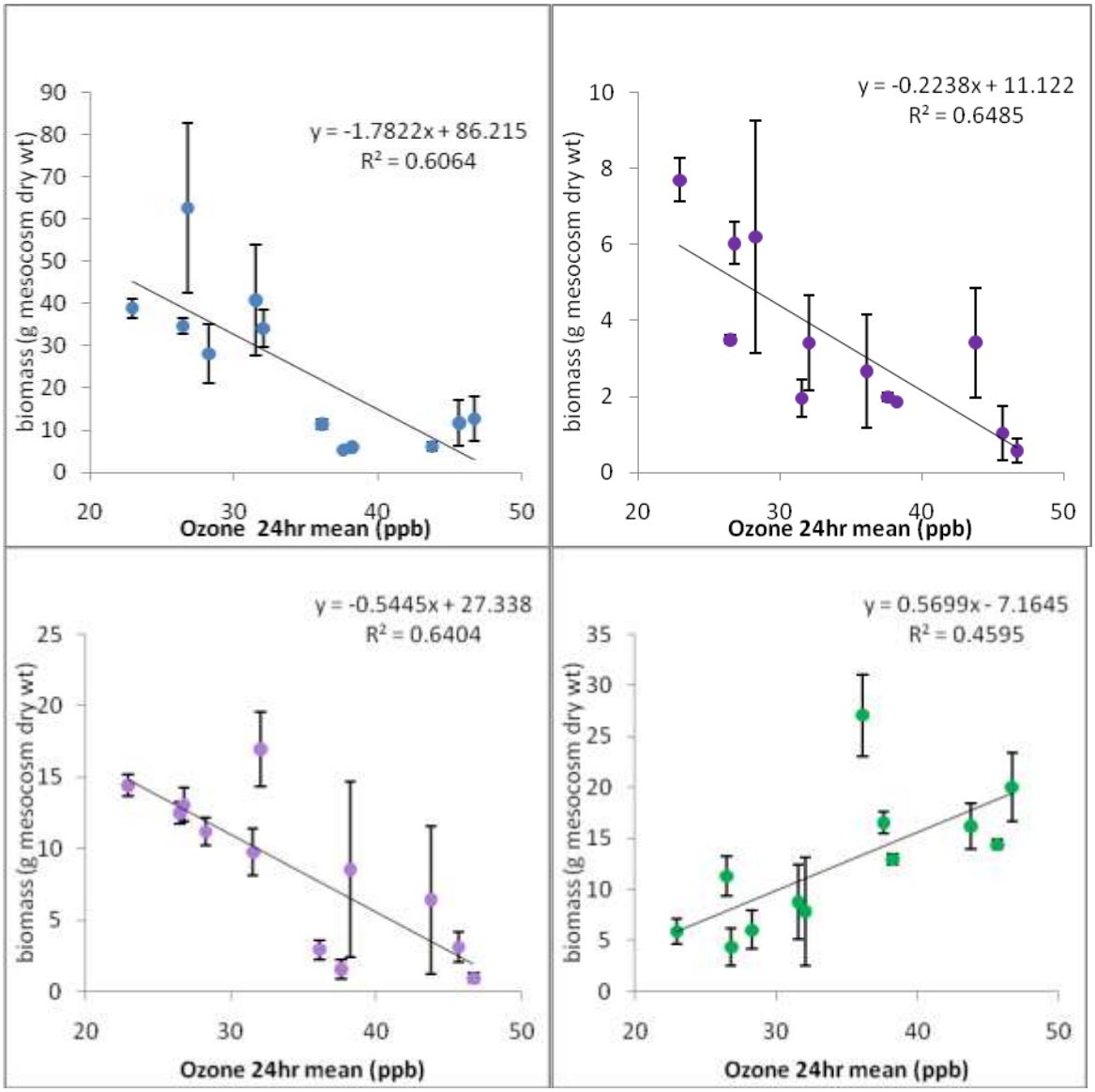


Figure 15. Exposure-response relationships of key components of a simulated SD9b sand dune community following 900 days' exposure to controlled levels of ozone. a) *Ononis repens* b) *Lathyrus pratensis* c) *Vicia* spp d) all grass species.

e) Effects on the hemi-parasite Rhinanthus minor

Efforts to establish the impacts of ozone on the hemi-parasitic annual *Rhinanthus minor* in experiments planned to encompass two host grass species of contrasting ozone sensitivity were aborted twice because of poor seed viability of the hemi-parasite due to mild winter conditions. However, over the past winter (2009/10), at the third time of trying, mature stands of both ozone-resistant *Lolium perenne* and ozone-sensitive *Phleum pratense* have been established in which there appears to be vigorous growth and germination of *R. minor*, so the delayed study is now programmed for entry into the Newcastle-based OTCs in Year 5 of the experimental programme.

Effects of long-term ozone exposure on competition

The sixth-year harvest performed during the course of study at Newcastle examining the effects of a simulated 2050 Upland ozone climate on species-rich UK upland meadow community (*Anthoxanthum odoratum-Geranium sylvaticum* [*Briza media* sub-community]; NVC MG3b) continued to reveal disturbing effects of elevated ozone levels on this high conservation value upland plant community (Figure 16), with a steady and consistent decline (statistically significant) in key species (eg. *Briza media* and *Lotus corniculatus*) balanced by an increase in the prevalence of competitive grasses (eg. *Alopecurus pratensis*). The decline in *Briza media* and *Phleum bertolonii* is of such magnitude that ozone-induced extinction of these species from this community is a possibility as the experiment progresses. If this was to be the case, then there would be an ozone-driven requirement to re-classify the NVC vegetation category for this community.

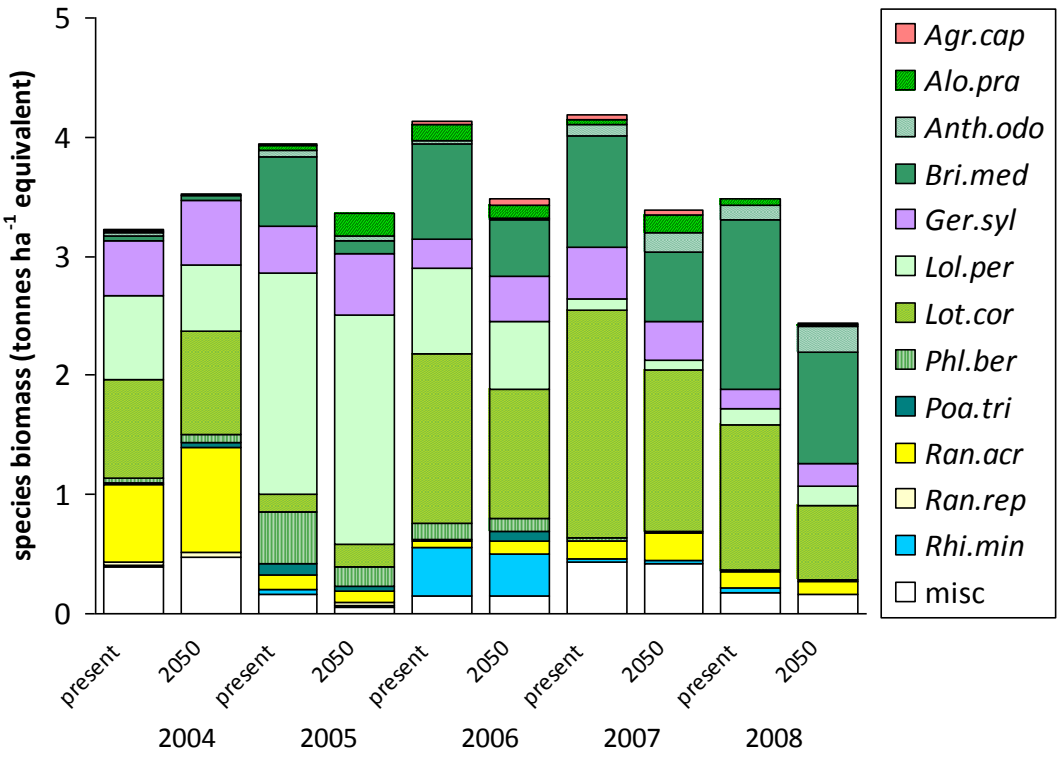


Figure 16. Impacts of simulated present-day versus 2050 UK-upland ozone climate on a mesotrophic grassland community (*Anthoxanthum odoratum - Geranium sylvaticum; Briza media* sub-community; NVC - MG3b) over five consecutive years. * indicate significant (P< 0.05) ozone-induced changes within a season for individual species in the community.

Effects on ecosystem processes

Effects of ozone on below-ground structure and function

This work package focussed on the below-ground characterization of MG3b grassland mesocosms, in close collaboration between the universities of York and Newcastle. This was because of the long duration of ozone exposure and the clear evidence of effects of ozone that have been found on composition, based on above-ground biomass, and because of the importance of mycorrhizas in nutrient acquisition in these low fertility grasslands. Effects of two ozone treatments (30 ppb and 50 ppb) were examined along with the interaction with different fertility levels (high and low residual P) within these treatments. Work on peatland mesocosms has continued under a parallel NERC grant. Copies of the final report to NERC of this work, including any policy implications, will be provided to DEFRA when it is completed in 2011.

Effects on below-ground biomass and composition

Soil cores were taken on 6th July 2009 (immediately prior to the above-ground harvest) and on 31st August 2009 (after regrowth). Roots were extracted from these cores (0-5 cm, and 5 cm up to maximum of 10 cm) and total root biomass was measured. No significant effects of ozone or fertility level were found on either sampling date. These findings are in contrast to the effects of ozone on total above-ground biomass that have been found in these mesocosms. As described above, there have been long-term shifts in species composition of above-ground biomass in these mesocosms. This raises the question of whether the species composition of the below-ground component is also affected by ozone. However, no previous studies have considered the effects of ozone on species composition below-ground in complex communities, because of the lack of appropriate measurement techniques. Using molecular methods, we plan to determine the abundance of roots of the different species in these samples, which will then allow us to compare the effects of ozone on species abundance above- and below-ground. This complex additional work, funded through other sources, is outside the scope of this final report, and is still in progress. We will report the results of this work to Defra when it is completed.

Overall mycorrhizal colonisation was measured in the same root samples that were used to assess effects on total root biomass. The proportion of individual roots that were colonised by mycorrhizas was determined in these samples, with a special focus on arbuscular mycorrhizas, which are vital to nutrient acquisition. The effects of ozone were relatively small, but statistical analysis combining the data for the two sampling dates showed a significant overall effect of ozone (P<0.01) in increasing both total and arbuscular mycorrhizal colonisation. In

July, it appeared that ozone increased overall colonisation in the low fertility, but not the high fertility treatment but no significant interaction between ozone and soil fertility treatments was found. Overall, there was little evidence of adverse effects of ozone below ground to mirror the reduced above-ground biomass and altered species composition above-ground that have resulted from long-term exposure to elevated background ozone concentrations.

Soil cores (bulk soil) were extracted from replicate mesocosms exposed long-term to 30 ppb or 50 ppb ozone. DNA was extracted from 3 horizons, amplified by PCR, subjected to DGGE then PCR products isolated and sequenced. 16S and 28S sequences were identified by NCBI BLAST search. The study revealed highly significant effects of the long-term ozone exposure on both the size and diversity of bacterial and fungal populations. The study highlights the potential scale of, as yet unstudied, below-ground ozone impacts (Figure 17). It is vital to establish the potential consequences of such impacts on ecosystem functioning. The loss of, for example, white rot fungi in grasslands exposed to elevated levels of ozone could have profound consequences as this component of the fungal community possesses the unique ability to degrade lignins which are a major fraction of the material that becomes soil humus.

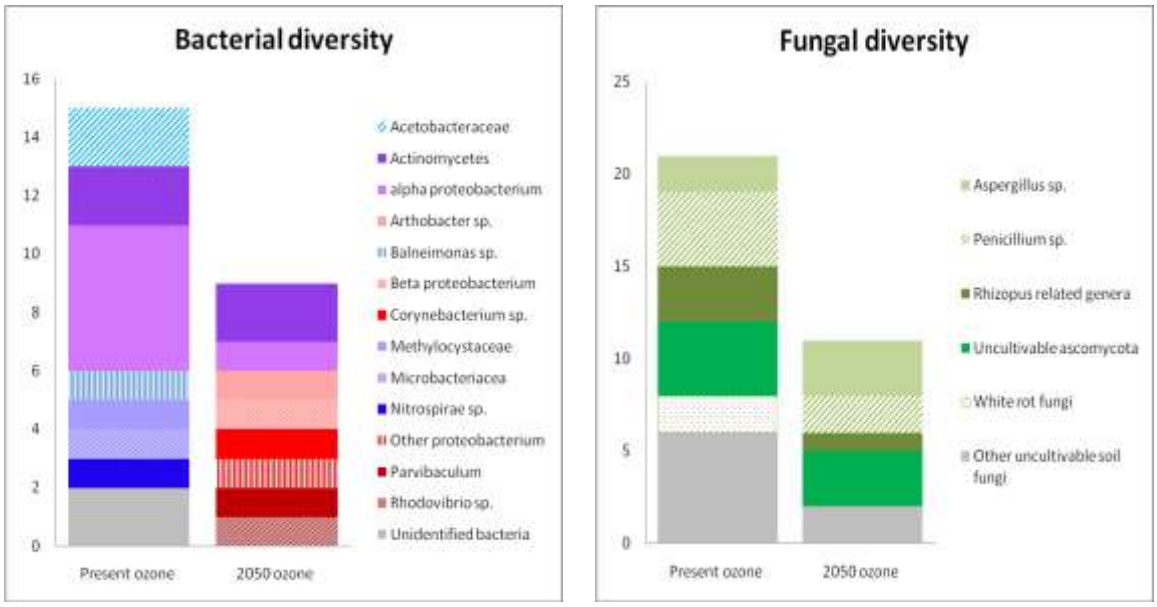


Figure 17. Impacts of long-term exposure to simulated present-day versus 2050 UK-upland ozone climate on the size and diversity of rhizosphere microflora supporting a mesotrophic grassland community (*Anthoxanthum odoratum* - *Geranium sylvaticum*; *Briza media* sub-community; NVC - MG3b).

Carbon fluxes

Changes in ozone exposure may have effects on carbon fluxes through reduced growth above- and below-ground and through changes in soil fluxes which are caused by changes in plant and microbial activity. Most studies of ozone effects on carbon fluxes have focussed on forests, which have the greatest above-ground carbon store. Much less is known about effects on carbon fluxes for grasslands and peatlands, both of which have important below-ground carbon stores. The reduced root growth and increased senescence observed in several experiments at CEH Bangor (see above) combined with effects on below ground microflora (Figure 17) indicates that below-ground carbon storage could potentially be reduced at ozone concentrations just above current ambient levels.

Experiments with lowland bog mesocosms in the Newcastle-based OTCs showed a significant reduction in methane fluxes during the summer, but not during the winter, when ozone exposure was low (summer mean concentration raised by 38 and 60 ppb above ambient in the two experimental years). In 2008, a new experiment with an exposure-response design was initiated using a different community from the Isle of Skye. The results (Figure 18) show that, within one season, there was a clear exposure-related effect with, as in the first experiment, methane emissions being reduced with increasing ozone concentrations; although the effect of adding 10 ppb ozone was not significantly different from the control treatment, it was consistent with there being little or no threshold for this effect.

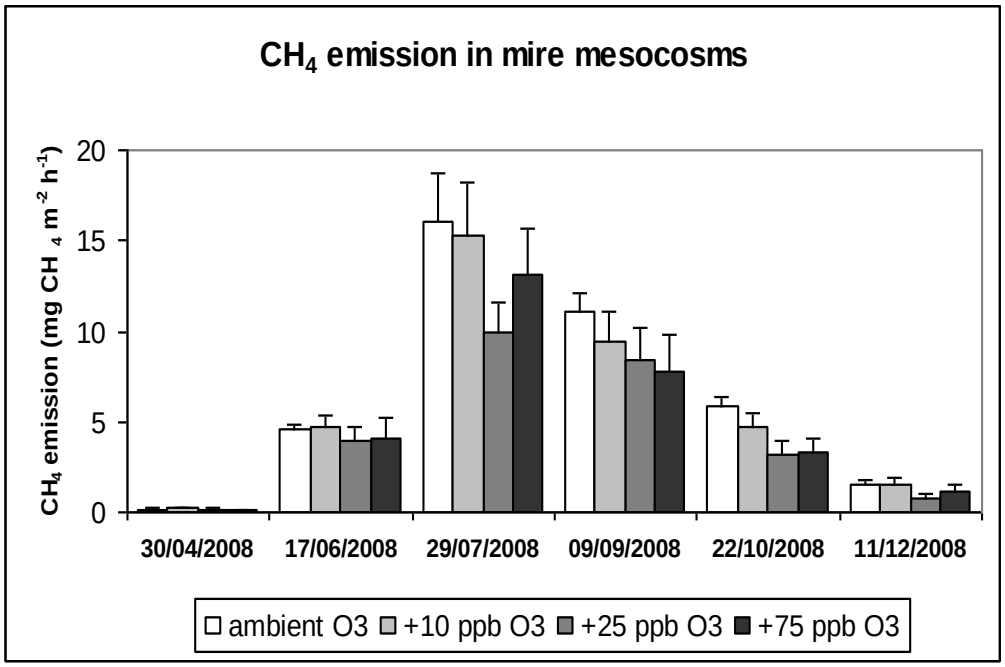


Figure 18. Effect of ozone treatment (expressed as mean concentration above ambient) on methane emissions from mire mesocosms established in the spring of 2008.

Experiments with wetland cores in the Bangor chambers produced different results, with a tendency for an increase in methane emissions in response to ozone. The reasons for these different experimental responses are uncertain but may be linked to differences in species composition in the two studies, and hence different routes for methane production and transport. There is evidence from experiments linked to the York OTC study that the effect of ozone is mediated by suppression of microbial activity, since litter incubated in soil water taken from the ozone treated mesocosms had much lower rates of microbial respiration, whereas there was no effect of the treatment received by the litter. No effects of ozone on dark ecosystem respiration was evident in either of the OTC experiments.

Water vapour fluxes

Results described in detail below indicate that ozone affects stomatal functioning via an effect on stomatal control by plant hormones. Plants are less able to respond to drought by closing stomata and thus lose more water during soil drying. With more frequent droughts predicted in the future this effect could exacerbate water conservation measures.

Soil water chemical composition

During the MG3b study pore water was sampled at approximately monthly intervals using Rhizon samplers. The samplers were analysed for DOC, ammonium and nitrate concentrations and the pH was recorded. The data show no evidence of significant effects of long-term ozone treatment on soil solution chemistry, a result that is consistent with the seeming lack of effects of ozone on root biomass.

Physiological Responses to Background Ozone Concentrations and Biological Factors/Mechanisms Controlling Sensitivity/Responses (Objectives 4 & 5)

Within air quality policy development there is a tendency to consider each pollutant individually, without consideration of associated biotic or abiotic stresses that might interact with the pollutant. Such stress may modify the response of vegetation to the pollutant or vice versa. Here we consider two stresses that often co-occur, and are increasingly likely to co-occur within our changing climate: ozone pollution and drought. Understanding the mechanisms behind the combined responses to both stresses will improve the reliability of flux-based risk assessments for (semi-)natural vegetation under current and future climatic conditions, provide possible biomarkers for rapid screening for ozone sensitivity, and allow development of tools for remediation in the field. Both drought and tropospheric ozone pollution can affect stomatal aperture and thereby the amount of water that plants lose/retain. Soil drying tightly controls stomatal aperture, often via production and altered accumulation of plant hormones such as abscisic acid (ABA; Wilkinson and Davies 2002). An increase in the amount of ABA sent up to the leaf signals the stomata to close. However the mechanisms that control stomatal aperture as atmospheric ozone levels fluctuate are less well understood. Despite much evidence in the literature that ozone can induce stomatal closure (e.g. Wittig et al 2007), several investigations have shown that stomata of some species close less sensitively in response to drought in ozone polluted areas or when ozone concentrations are experimentally elevated in the laboratory or near-field conditions (work conducted under this contract, described below and published in Mills et al 2009, Wilkinson and Davies, 2009). Species which close their stomata

persistently in elevated ozone will exhibit reduced biomass (reduced supply of nutrients and other growth promoting factors via the transpiration stream). Species in which stomata remain more open under drought (soil drying, high temperature, high VPD) and ozone pollution will run the risk of dehydration. Both impediments will impact on competitive potential/abundance. Whilst we know something about the mechanisms that may contribute to ozone-induced stomatal closure, those whereby elevated ozone reduces stomatal sensitivity to drought have not been described.

Here we assess effects of ozone concentration on plant hormone production and on stomatal and growth responses to soil and plant water status and to the major drought response hormone ABA (objective 4). By understanding how ozone-induced damage occurs we have been able to show that its amelioration is possible (objective 5). Ozone was elevated to a range of concentrations encompassing current and predicted average background concentrations (Meehl et al., 2007), which were, however, within the range of current UK ambient maxima (Hayes et al., 2007). Fumigation of plants in mesocosms or pots was carried out in solar domes at CEH Bangor or in 1.0 x 1.0 m2 cabinets inside a glasshouse at Lancaster.

Results and formulation of hypotheses

We studied stomatal responses to elevated ozone in several species from a UK upland grassland community and determined that for the most part stomata either opened in well-watered plants (Figure 19) or closed less sensitively in response to a) dehydration of detached leaves (Figure 20); b) soil drying regimes (Figure 21); and c) direct applications of abscisic acid (ABA) (Figure 22). Loss of stomatal sensitivity to ABA occurred whether the hormone was synthesized endogenously, or whether it was supplied to the xylem of detached leaves via the petiole, as a surface spray to the foliage of intact plants, or as an injection to the midrib of leaves on the intact plant. This response could be observed 2 days after exposure of plants to ozone in some cases, and for up to 4 months (longest time tested). Leaf growth was enhanced in well-watered and drying soil in elevated ozone over a period of 8 weeks exposure (*Anthoxanthum odoratum*). Elevated ozone had no effect on endogenous ABA concentrations.

Up-regulation of ethylene synthesis during ozone exposure is a common response in ozone sensitive species/varieties. Tanaka et al. (2005, 2006) have determined that exogenously supplied ethylene has an antagonistic effect on ABA-induced stomatal closure in unpolluted *Arabidopsis*. We proposed that ozone-induced ethylene production could cause the observed antagonism of the stomatal response to ABA in ozonated *L. hispidus*, and showed that ethylene generation by *L. hispidus* leaf tissue was indeed increased by ozone exposure over 14 days. When intact plants were pre-treated with 1-MCP, a compound which inhibits ethylene from binding to its receptors, the ability of ABA (2.5x10-6M – intact plants sprayed daily for 3 days prior to measurement) to close stomata was restored in ozone-exposed plants. Similar effects were seen when soil was inoculated with concentrated suspensions of cultures of naturally occurring rhizobacteria that produce ACC deaminase (ACCd - Belimov et al, 2009). ACC is the ethylene precursor, and ACCd reduces its concentration in the xylem sap. Soil inoculation with *Alcaligenes xylosoxidans* strain Cm4 reduced ethylene emission from leaf tissue and restored the stomatal response to ABA in ozone-treated *L. hispidus* 1-3 weeks later.

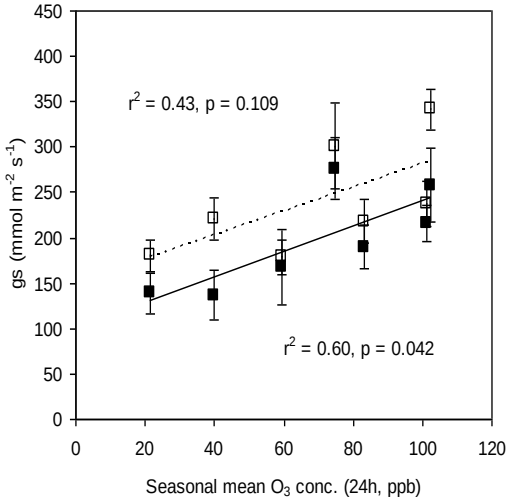


Figure 19: Stomatal conductance in response to ozone

□ = *Dactylis glomerata*, ■ = *Leontodon hispidus. (*Mills et al., 2009).

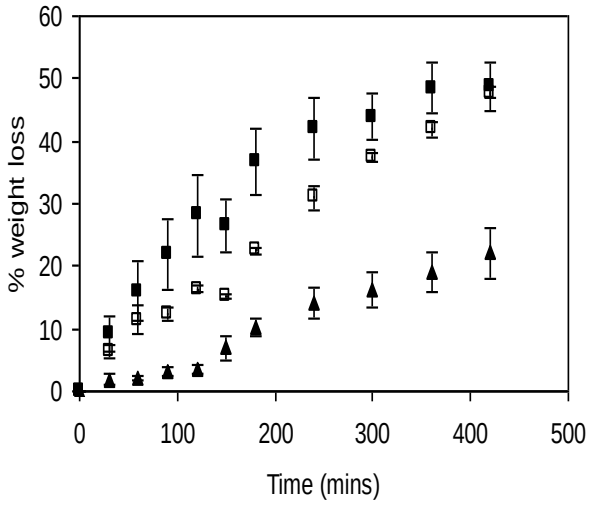


Figure 20: loss of water through open stomata of *D. glomerata* in response to ozone. ■ = ambient air ozone concentration, AA, -20 ppb; □ = AA +24 ppb; Δ = AA+60 ppb *(*Mills et al., 2009).

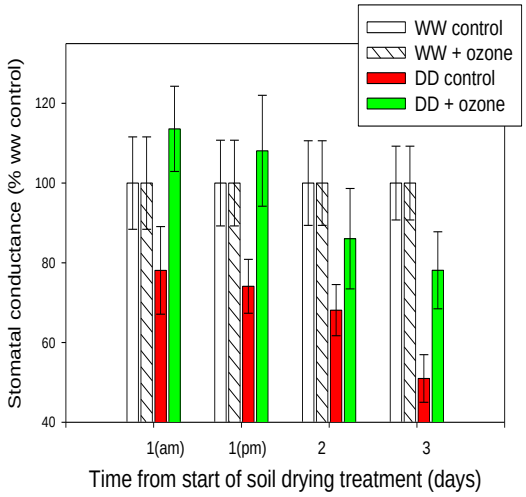


Figure 21: stomatal conductance relative to control in *L. hispidus* subjected to well-watered (WW) or soil drying (DD) treatments.

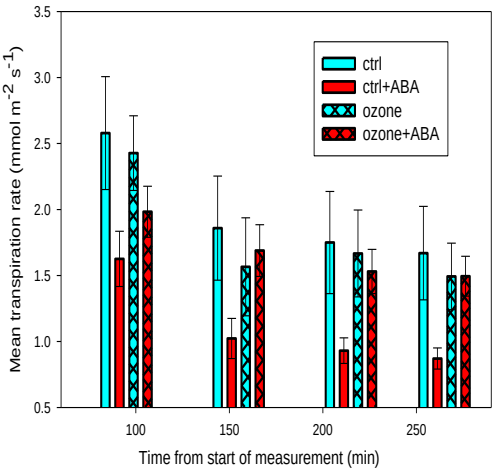


Figure 22: transpiration rates from *L. hispidus* exposed to ozone with/without ABA (Wilkinson and Davies, 2009)

Figure 23 shows that when the soil around *L. hispidus* plants was allowed to dry, stomata in plants in elevated ozone remained more open, as expected, whilst those in ozonated 1-MCP treated plants closed as sensitively as those in ambient ozone. This indicates that when ethylene is prevented from binding to its receptors, stomata regain their sensitivity to the ABA that is synthesized by the plants in the drying soil.

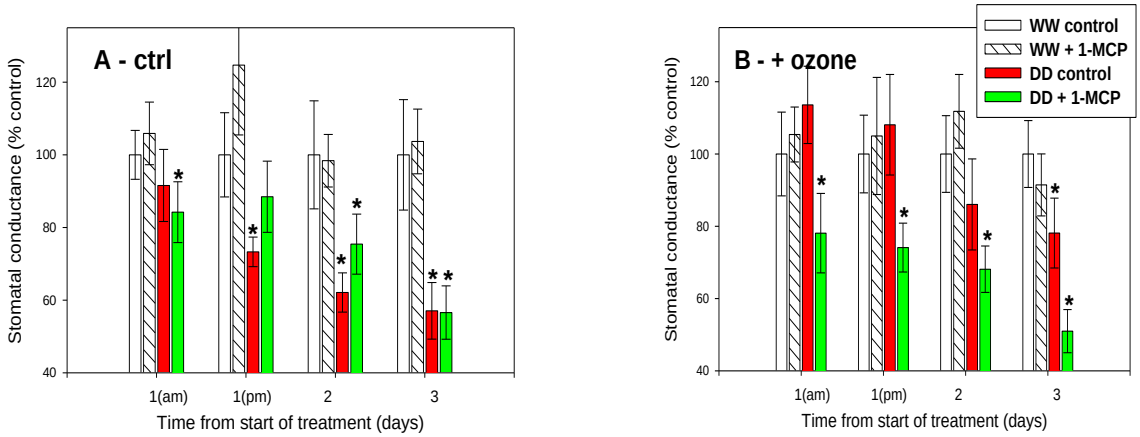


Figure 23: Stomatal conductance in *L. hispidus* in response to ozone and soil drying (DD) with and without addition of 1-MCP which inhibits ethylene from binding to its receptors. Ethylene inhibition significantly reduced stomatal conductance in ozone-treated plants under soil drying, but had no overall effect on plants without ozone.

In the final year of the project we have extended these studies to ozone-sensitive and ozone-tolerant clover (Figure 24), showing that under droughted conditions an ozone-sensitive accession of clover has more open stomata in polluted air than an ozone-tolerant accession, the implication being that this accession will lose too much water to sustain growth, and that this is the reason for the differences in sensitivity between the two types of clover.

Ethylene antagonises the stomatal response to ABA, and early indications have been that the same is true of shoot growth (see Wilkinson & Davies 2010). This implies that improvements in the tolerance of a particular species to ozone could be implemented using the field-approved ethylene perception inhibitor 1-MCP, or other field-viable treatments that reduce ethylene production, such as soil seeding with ACC-deaminase-producing rhizobacteria (see Wilkinson & Hartung 2009 and see below). First it is necessary to screen species for ozone sensitivity on this basis, and this entails assessing the efficacy of screens based on a) stomatal sensitivity to applied ABA, and/or b) the extent of ethylene emission from leaf tissue.

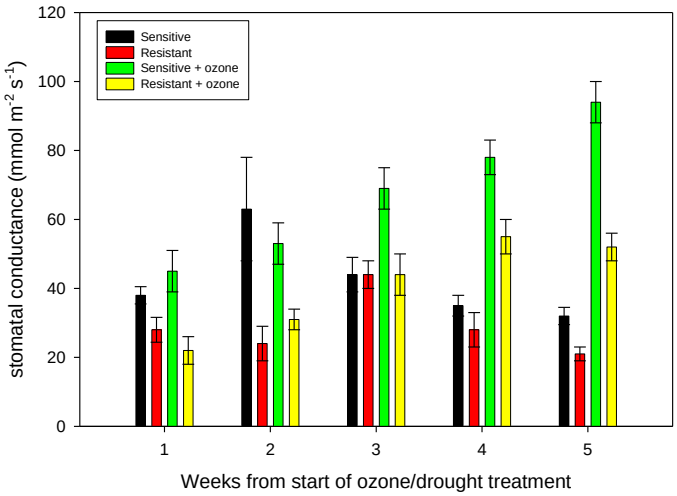


Figure 24: stomatal conductance in sensitive (black) and tolerant (red) clover, exposed to ozone (sensitive=green, tolerant=yellow), showing much greater water loss of sensitive clover exposed to ozone.

Plant screening

Tests for sensitivity to ABA and extent of ethylene emission have been carried out in several grassland species under predicted background ozone concentrations for 2075 (approximately 70 ppb), based on ground cover at the Keenley Fell field fumigation site: *Anthoxanthum odoratum*, *Holcus lanatus, Leontodon hispidus, Plantago lanceolata, Trifolium repens.* It is assumed that species in which more ethylene is emitted will be those that a) are less sensitive to ABA with respect to stomatal closure in lab-based experiments, and/or have more closed stomata under well-watered low ABA conditions, and b) exhibit reduced biomass at the Keenley field fumigation site. Furthermore, effects of ethylene +/- ABA have been tested in model species (tomato and lettuce) to study these relationships in greater depth. Ultimately this will enable us to predict effects of ozone pollution on vegetation health in other communities / crop species without the necessity for further large-scale and expensive field fumigation trials, simply by assessing stomatal / ethylene-emitting responses in the laboratory.

Ozone increased growth in *Anthoxanthum odoratum* in the absence of ABA, and increased stomatal conductance (gs) both in the presence and absence of ABA. This mirrors effects seen on gs in *Dactylis glomerata* and *Leontodon hispidus* (see above). Ethylene emission from *Holcus lanatus* leaves was very high in both controls and ozone-treated plants. ABA did not reduce stomatal conductance in either controls or ozone-treated plants; conversely, it actually opened stomata in ozone-treated plants. Ozone reduced stomatal aperture in *Plantago lanceolata*, both in the presence and absence of ABA. Ethylene emission from leaves was greater in ozone treated plants.

Because ethylene has been reported to both open and close stomata (see Wilkinson and Davies 2009, 2010), and to reduce growth (see Wilkinson & Davies 2010), and because ethylene emission is a primary response to ozone, we decided to investigate effects of ethylene on stomatal aperture and growth in greater depth in unpolluted model species. Ethylene was generated in plants by supplying them with the ethylene precursor ACC via injection to the leaf petiole (tomato), or via a foliar spray application (tomato and lettuce). This treatment gave rise to the intended increase in ethylene. We hypothesized that ethylene can close stomata (as ozone does in well-watered plants of some species) in isolation, but that super-imposed on this effect is another whereby ethylene antagonises the stomatal response to ABA such that stomata remain more open (as induced by ozone in droughted *L.hispidus* plants and/or in those manipulated to contain more ABA). When the ethylene precursor ACC was injected/sprayed into/onto tomato or lettuce plants it closed stomata (reduced gs), however when both ACC and ABA were applied together, ACC re-opened stomata in comparison to those treated with ABA alone. An identical effect of ACC and ABA was shown for leaf growth. These data are highly novel, with wide-reaching implications in both polluted and non-polluted plants.

The many implications of these findings are discussed in Wilkinson and Davies (2010). These data allow us to predict that species in which ozone induces stomatal closure (Wittig et al. 2007) may do so via an up-regulation of ethylene, rather than simply as a secondary response to a reduction in photosynthesis. This seems to be the case in *Plantago lanceolata*. Thus a simple foliar spray with ABA may, ironically, re-open stomata and improve productivity in the field in certain ozone polluted crops / communities. This possibility is borne out by the results described above: in *Holcus lanatus* ABA unusually induced stomata to open, especially in polluted plants, and ethylene production from these plants was high (both in the presence and absence of ozone - probably due to the high ambient temperature of the glasshouse). However, ABA did not re-open stomata of *Plantago lanceolata* in

ozone, whilst ozone did up-regulate ethylene. Different species will have different sensitivities to both ethylene and ABA, and in some of the experiments on the model species there was a point at which the ABA concentration became too high for the ethylene to re-open the stomata / prevent their closure, and there was a point at which the ethylene concentration became too high for ABA to re-open stomata. In the case of *Anthoxanthum odoratum* ozone opened stomata in the absence of ABA as well as in its presence. It must be borne in mind that plants always contain some ABA even when they are not droughted (or treated with ABA exogenously). *Anthoxanthum*, like *Holcus,* is very sensitive to high temperature, and it seems to be likely that both growth and conductance were already below maximum, even in controls, as a result of a raised ABA concentration, and that ozone-induced ethylene may have overcome the effects of this ABA on growth and stomatal conductance to induce an increase in both parameters. Ethylene measurements are currently being conducted in this species.

It appears that the balance between ethylene and ABA is the most important determinant of effects of stresses such as ozone, combined with soil and air water deficit, on stomatal aperture, rather than the change in either hormone in isolation. This knowledge allows us to make much more accurate predictions of the effects of ozone on vegetation health.

Interactions below ground

A community also comprises the below-ground flora and fauna, which have also been shown to affect vegetation health above-ground (see Wilkinson & Hartung 2009). Soil/root interface-dwelling bacteria (rhizobacteria) often use plant-sourced products as a carbon source, and those that use the ethylene precursor ACC as a carbon source (via production of ACC-deaminase) may be of particular relevance with respect to the effect that they may have on plant ethylene production. An ozone-induced change in rhizobacterial community balance towards ACC-deaminase producing bacteria has been suggested by our early research in which ozone reduced ethylene production in bacterially treated plants (see below). Thus we studied changes in bacterial community structure under ozone to determine whether this can potentially either mitigate or augment an effect of ozone on plant ethylene production.

As described above for the mesocosm study at Newcastle, ozone can reduce rhizobacterial populations by as much as 50%. If this includes those bacteria that modulate plant ethylene production, then effects on ozone-induced ethylene generation will be exacerbated. This will undoubtedly feed into the extent to which a community is affected by ozone as regards its water and carbon balance, via effects on stomata and growth. We have developed a method to study the impact on ethylene production in the field. Bacterial community structure was analysed from the rhizosphere (soil attached to a shaken, uprooted plant) of a 5-week-old lettuce crop (June 2009), inoculated with and without *Variovorax paradoxus* 5C2 (ACC-deaminase producing rhizobacteria), and either well-watered or subjected to drought. DNA and RNA were extracted and thermal gradient gel electrophoresis (TGGE) was performed with the samples of total DNA from the rhizosphere. Total DNA extracts from soil samples were pooled before PCR. Primer sets EF4-EF3 were used for direct amplification of 16S-rDNA sequences from extracted lettuce rhizosphere DNA. A TGGE maxi system (Biometra) was used for sequence-specific separation of community PCR products corresponding to the V3 region of the bacterial 16S rRNA. All ozone treatments showed a similar species pattern and the numbers of individuals of each population (indicated by the density of the bands) was similar for all treatments. The only difference between treatments was that the band corresponding to *Variovorax paradoxus* 5C-2 was only detected in inoculated plots. This method will allow us to determine whether ozone affects the community structure of natural rhizobacterial populations, which can also be used to ascertain whether any of the changes pertain to ACC-deaminase producing species. This will be applied to the Keenley soil populations with and without ozone during the 2010 growing season.

Policy implications

Effects on individual species

Table 2 lists the species that have been shown to respond to ozone at concentrations (either long-term or seasonal average) that are below or at current-day exposures. Even without taking into account predicted chronic increases in 'background' ozone concentrations, these results show that present-day ozone concentrations are affecting species of conservation/biodiversity/aesthetic importance in the UK. Moreover, we have shown that effects on below-ground parts (roots, bulbs) may be significant even in the absence of obvious effects (whether as visible symptoms or reduced growth) above ground. Such effects are likely to be exacerbated in future, particularly if they are combined with increased risk of drought. We have also shown that individual species responses cannot be separated from the environment in which they are growing, especially the species with which they compete, and that effects on growth depend on the effective flux of ozone, which for low-growing species may be determined by the size and density of the canopy of other species in which they grow. We now have a better understanding of the interplay of plant hormones that control responses to both ozone and drought, and this has potential for screening semi-natural vegetation in the laboratory in order to predict responses to the combined stresses.

Effects on communities, especially BAP Habitats

We have shown that vegetation communities of conservation importance can be affected by chronic exposure to relatively low levels of ozone, leading to changes in species composition. In particular, species which are essential for type classification (e.g. *Briza media*) have been shown to be particularly at risk. Both mesocosm and field studies have shown that the hemi-parasite *Rhinanthus minor* responds to low levels of ozone, and therefore its role in suppressing the competitive growth of grasses in favour of forbs and legumes is diminished. The results from the field fumigation experiment at Keenley Fell have shown an unexpected decrease in the forb population, given the very small increment in ozone exposure at the site and only 3 years of exposure. Changes in senescence and flowering in the absence of any visible injury characteristic of acute ozone injury (e.g. leaf stippling) also point to the insidious effects of chronic low-level ozone exposure in producing slow changes in communities which would not necessarily be noticed in the absence of controlled experiments or detailed monitoring. Moreover, some of the largest effects have been seen below ground, both in changes in root systems, and as effects on microbial and fungal populations, which up to now have not been recognised.

Effects on ecosystem services

The benefits provided by natural ecosystems can be summarised by the term ecosystem services. To date, there has been little systematic assessment of the implications of ozone on the services provided by ecosystems in the UK. The research conducted under this project has provided evidence for effects on ecosystem services as summarised in Table 3 and delivered exposure thresholds for detectable adverse effects on a range of different vegetation types.

Table 3. Summary of effects of ozone on ecosystem services revealed in this project, showing the inferred impact and the evidence to support it.

Ecosystem service	Impact	Evidence
Food, fuel and fibre	Decreased production (-)	Reduced yield in long-term mesocosm experiments
	Decrease in forage quality (-)	Reduction in forb and legume components in species mixtures Enhanced senescence associated with decline in forage quality
Water flow and quality	Reduced in hot, dry periods (-)	Implied by loss of stomatal regulation of transpiration and reduced root growth
	Increased organic content (-)	Evidence of increased DOC levels in bog mesocosms
Climate regulation	Reduced below-ground C (-)	Implied by reduced root growth
	Shifts in methane fluxes (+/-)	Observed in wetland mesocosms
Genetic	Reduced cover and biomass of sensitive species (-)	In mixed species mesocosms, forb and legume species have been out-competed by grasses.
Pollination	Reduced flowering (-)	Observed in woodland plant species and as a carry-over effect of ozone on grassland forbs
Aesthetic	Loss of characteristic and attractive species (-)	In long-term MG3b mesocosm and woodland bulb studies, there have been adverse effects on attractive and characteristic species. Attractive forbs out-competed by grasses in mesocosms.
	"Early autumn" (-)	Premature yellowing of grasslands due to enhanced senescence

Improvements to understanding and implications for modelling/ prediction/mitigation

Greater understanding of the basis of genotypic sensitivity to ozone (stomatal sensitivity to combinations of ABA and ethylene) will allow us a) to predict effects of ozone on community structure / to identify "at risk" agricultural crops, using lab-based screening trials, and b) to determine which communities/crops will benefit from ozone mitigation protocols (chemical / rhizobacterial treatments, choice of species make-up in managed pasture, crop breeding to reduce ethylene / ACC synthesis, policy decisions to control pollutant emission).

These investigations of the physiological impacts of ozone on (semi-) natural vegetation have revealed subtle effects on stomatal functioning in apparently "healthy" leaves that reduce the capacity of plants to respond to drought, a stress which frequently co-occurs with episodes of ozone pollution. This challenges the widely accepted view that ozone always induces stomatal closure (e.g. Wittig et al. 2007). Such effects have been detected experimentally in the laboratory and also after long-term exposure to increasing background ozone concentrations in near-field conditions within solardomes. Through a variety of experimental approaches, we have shown that ozone stress induces plants to release the hormone ethylene, which in turn reduces the capacity of plants to respond to the plant hormone abscisic acid – a hormone that induces stomatal closure and reductions in shoot growth as soil dries (objective 4). This renders plants incapable of responding to drought, and may

ultimately result in increased dehydration, enhanced senescence and reduced productivity. These responses can occur at ozone concentrations that are within current UK ambient summer peaks.

Thus we have shown that current and future predictions of ozone impacts in a changing climate need to take into consideration the frequency and extent of episodes of drought. By understanding these mechanisms we also provide evidence that 1-MCP and rhizobacteria (and other treatments that reduce ethylene production/perception) can be used as tools to ameliorate ethylene-based damage induced by ozone (objective 5), as the use of these treatments can be scaled up to field level. It may also be possible to use the extent of ozone-induced ethylene production as a biomarker for ozone sensitivity.

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References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

Published

Cape J.N., Bassin S., Fuhrer J., Gerosa G., Alonso R., Gimeno B., Gruenhage L., Mikkelsen T.N., Mills G., 2008. Challenges in understanding the risks to natural and semi-natural vegetation from ozone exposure. Italian Journal of Agronomy 3, 53-59

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Submitted

Hayes, F, Mills, G, Jones, L, Ashmore M. Does a simulated upland grassland community respond to increasing background, peak or accumulated exposure of ozone? Submitted to Atmospheric Environment in December 2009.

In preparation

Hayes, F., Mills, G., Harmens, H., Wyness, K. Carry-over effects of increasing background ozone on *Leontodon hispidus* in competition with *Dactylis glomerata* or *Anthoxanthum odoratum*

Presented

Ozone Critical Levels Workshop, Ispra, November 2009 Hayes, F., Mills, G. Flux-effect relationships for effects on above- and below-ground carbon storage and shifts in species balance.